

1983

THE TOXICOLOGICAL SIGNIFICANCE AND ETIOLOGY OF THE DIELDRIN (HEOD)-INDUCED HYPERGLYCEMIC RESPONSE IN THE IMMATURE AND ADULT RAT.

GLYN ROBERT. FOX
University of Windsor

Follow this and additional works at: <http://scholar.uwindsor.ca/etd>

Recommended Citation

FOX, GLYN ROBERT., "THE TOXICOLOGICAL SIGNIFICANCE AND ETIOLOGY OF THE DIELDRIN (HEOD)-INDUCED HYPERGLYCEMIC RESPONSE IN THE IMMATURE AND ADULT RAT." (1983). *Electronic Theses and Dissertations*. Paper 4320.

This online database contains the full-text of PhD dissertations and Masters' theses of University of Windsor students from 1954 forward. These documents are made available for personal study and research purposes only, in accordance with the Canadian Copyright Act and the Creative Commons license—CC BY-NC-ND (Attribution, Non-Commercial, No Derivative Works). Under this license, works must always be attributed to the copyright holder (original author), cannot be used for any commercial purposes, and may not be altered. Any other use would require the permission of the copyright holder. Students may inquire about withdrawing their dissertation and/or thesis from this database. For additional inquiries, please contact the repository administrator via email (scholarship@uwindsor.ca) or by telephone at 519-253-3000ext. 3208.

CANADIAN THESES ON MICROFICHE

I.S.B.N.

THESES CANADIENNES SUR MICROFICHE



National Library of Canada
Collections Development Branch

Canadian Theses on
Microfiche Service

Ottawa, Canada
K1A 0N4

Bibliothèque nationale du Canada
Direction du développement des collections

Service des thèses canadiennes
sur microfiche

NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us a poor photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30. Please read the authorization forms which accompany this thesis.

AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de mauvaise qualité.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30. Veuillez prendre connaissance des formules d'autorisation qui accompagnent cette thèse.

THIS DISSERTATION
HAS BEEN MICROFILMED
EXACTLY AS RECEIVED

LA THÈSE A ÉTÉ
MICROFILMÉE TELLE QUE
NOUS L'AVONS REÇUE

THE TOXICOLOGICAL SIGNIFICANCE AND ETIOLOGY OF THE
DIELDRIN (HEOD)-INDUCED HYPERGLYCEMIC RESPONSE IN THE
IMMATURE AND ADULT RAT

by

© Glyn Robert Fox

A Dissertation submitted to the
Faculty of Graduate Studies and Research through
the Department of Biology in Partial Fulfillment of the
requirements for the Degree of Doctor of Philosophy at the
University of Windsor.

Windsor, Ontario, Canada

1983

Glyn Robert Fox

©

1983

All Rights Reserved

793335

ABSTRACT

THE TOXICOLOGICAL SIGNIFICANCE AND ETIOLOGY OF THE DIELDRIN (HEOD)-INDUCED HYPERGLYCEMIC RESPONSE IN THE IMMATURE AND ADULT RAT

by

Glyn Robert Fox

Carbohydrate metabolism was studied in rats given 1 LD₅₀ of HEOD. Plasma glucose was elevated in five day old rats, (17% at one hour) and in adults (45 and 38% at one and three hours). Plasma FFA were decreased only in five day old rats (9% at one hour). Hepatic glycogen was reduced in five and ten day old rats only, (22 and 16% at one hour). Thus, dieldrin does not alter carbohydrate metabolism in immature rats, but causes hyperglycemia in adults.

Hepatic fructose-1,6-diphosphatase activity was elevated (17% at one hour) in five and decreased (18% at three hours) in ten day old rats. Phosphoenolpyruvate carboxykinase (PEPCK) activity was increased (38%) in adults at one hour, and in all ages, at three hours (56 - 115%). Glucose-6-phosphatase activity was unaltered. Thus, only PEPCK activity is significantly increased by dieldrin.

In ten day old rats given HEOD, d-glucose (6 g/kg p.o.) reduced mortality (42%). Thus, elevated plasma glucose protects dieldrin-exposed pups. Plasma glucose maintained at 2-3 times normal by d-glucose (6 g/kg p.o.) or by 2-deoxyglucose (1 g/kg s.c.) did not affect HEOD toxicity in adults. Thus, potentiating HEOD-induced hyperglycemia is not adaptive

in the adult.

Insulin (2 U/kg s.c.) caused hypoglycemia, decreased hepatic glycogen (79%) and increased PEPCK activity (130%) and raised mortality (46%) in adults given HEOD. Thus insulin-induced hypoglycemia potentiates the acute toxicity of HEOD.

In adults given HEOD and 40 mg/kg i.p. phenobarbital, hyperglycemia and mortality were reduced 41 and 80%. Toxicity was restored by increasing the hyperglycemia (41%) with d-glucose (6 g/kg p.o.). In dieldrin and insulin (2 U/kg s.c.) treated rats, phenobarbital increased hypoglycemia (35%) and decreased mortality (34%). Thus, HEOD-induced hyperglycemia is of CNS origin and may be toxic.

Atropine (4 mg/kg s.c.), given with HEOD to adults prevented hyperglycemia for four hours and reduced mortality (42%). Atropine given at 4-18 hours after HEOD had no effect. L-a-methyldopa (200 mg/kg i.p.) in adults, decreased plasma glucose (24%) for two hours, but did not change HEOD toxicity. d,l-propranolol (8 mg/kg s.c.) did not alter the effects of HEOD.

Thus, we suggest that HEOD-induces CNS stimulation and increases plasma glucose by eliciting pancreatic glucagon release, via increased parasympathetic outflow. Also an α -adrenergic inhibition of insulin release may also occur. The β -adrenergic system plays no role in HEOD-induced hyperglycemia.

For the 1200 plus.

ACKNOWLEDGEMENTS

I wish to express my gratitude to the members of my committee for their useful comments and criticisms. A special thanks is extended to Dr. B.B. Virgo for supervision, and assistance far above the call of duty. I must also gratefully acknowledge Vivian's aid and support without which this work would not have been completed.

TABLE OF CONTENTS

| | page |
|--|------|
| ABSTRACT..... | iv |
| DEDICATION..... | vi |
| ACKNOWLEDGMENTS..... | vii |
| LIST OF TABLES..... | xiii |
| LIST OF FIGURES..... | xvii |
| LIST OF APPENDICES..... | xix |
| INTRODUCTION..... | 1 |
| LITERATURE REVIEW | |
| Chlorinated hydrocarbon insecticides - Effects on carbohydrate metabolism..... | 5 |
| A. The adult rat..... | 5 |
| B. The neonate..... | 34 |
| C. Adult animals other than the rat..... | 37 |
| MATERIALS AND METHODS | |
| A. General materials and methods..... | 48 |
| Ai. Chemicals..... | 48 |
| Aii. Technical notes and procedures common to all experiments..... | 48 |
| Aiii. Animals..... | 41 |
| B. Acute toxicity of HEOD in: 5, 10 and 60 day old rats..... | 41 |
| Bi. HEOD LD50 determination in five and ten day old pups..... | 42 |
| Bii. HEOD LD50 determination in 60 day old males..... | 43 |
| C. Determination of: plasma glucose, plasma free fatty acids and hepatic glycogen, one and three hours after HEOD..... | 44 |
| Ci. Five and ten day old pups - sample collection..... | 45 |

| | | |
|-------|--|----|
| Cii. | Adult males - sample collection..... | 45 |
| Ciii. | Analytical procedures..... | 45 |
| a) | Plasma glucose..... | 45 |
| b) | Plasma FFA..... | 46 |
| c) | Hepatic glycogen..... | 47 |
| D. | Determination of gluconeogenic enzyme activities in rat liver..... | 48 |
| Di. | Hepatic enzyme studies - sample collection..... | 48 |
| Dii. | Hepatic enzyme studies - quantification..... | 50 |
| a) | Fructose-1,6-diphosphatase..... | 50 |
| b) | Glucose-6-phosphatase..... | 51 |
| c) | Phosphoenolpyruvate carboxykinase..... | 52 |
| E. | Effects of exogenous d-glucose on plasma glucose and mortality in HEOD-treated rats..... | 57 |
| Ei. | Effects in adult males..... | 57 |
| Eii. | Effects in ten day old pups..... | 62 |
| F. | Effects of 2-deoxyglucose on: plasma glucose, convulsion onset latency time and mortality in HEOD-treated male rats..... | 63 |
| G. | Effects of insulin on: plasma glucose, hepatic glycogen and PEPCK activity, mortality and convulsion onset latency time in HEOD-treated male rats..... | 64 |
| H. | Effects of phenobarbital on: plasma glucose, hepatic glycogen and PEPCK activity, mortality and convulsion onset latency time in HEOD-treated male rats..... | 65 |
| I. | Effects of phenobarbital and exogenous glucose on: plasma glucose, hepatic glycogen and PEPCK activity, mortality and convulsion onset latency time in HEOD-treated male rats..... | 66 |
| J. | Effects of phenobarbital and insulin on: plasma glucose, hepatic glycogen and PEPCK activity, mortality and convulsion onset latency time in HEOD-treated male rats..... | 66 |
| K. | Effects of atropine on: plasma glucose, hepatic glycogen and PEPCK activity, mortality and convulsion onset latency time in HEOD-treated male rats..... | 67 |
| L. | Effects of L-a-methyldopa on: plasma glucose, hepatic glycogen, PEPCK and G6P activities, mortality and convulsion onset latency time in HEOD-treated male rats..... | 68 |

| | | |
|----|--|----|
| M. | Effects of d-1-propranolol on: plasma glucose, hepatic glycogen and PEPCK activity, mortality and convulsion onset latency time in HEOD-treated male rats..... | 69 |
| N. | Methods of statistical analysis..... | 70 |

RESULTS

| | | |
|-------|--|-----|
| A. | Age-specific LD50 for dieldrin..... | 71 |
| B. | Effect of dieldrin on energy substrates..... | 71 |
| Bi. | Five day old pups..... | 71 |
| Bii. | Ten day old pups..... | 73 |
| Biii. | Sixty day old adult males..... | 76 |
| C. | Effects of dieldrin on the activities of hepatic gluconeogenic enzymes..... | 79 |
| Ci. | Plasma glucose..... | 79 |
| Cii. | Hepatic fructose-1,6-diphosphatase activity..... | 82 |
| Ciii. | Hepatic glucose-6-phosphatase activity..... | 86 |
| Civ. | Hepatic phosphoenolpyruvate carboxykinase activity..... | 86 |
| Cv. | Relevance of enzyme study results..... | 89 |
| D. | Exogenous d-glucose effects in HEOD treated rats.... | 90 |
| Di. | Adult males..... | 90 |
| Dii. | Ten day old pups..... | 94 |
| E. | Insulin: effects in HEOD-treated adult rats..... | 96 |
| F. | Phenobarbital: effects in HEOD-treated adult rats.. | 103 |
| G. | Exogenous d-glucose and phenobarbital: effects in HEOD-treated adult rats..... | 109 |
| H. | Insulin and phenobarbital: effects in HEOD-treated adult rats..... | 114 |
| I. | 2-Deoxyglucose: effects in HEOD-treated adult rats. | 122 |
| J. | Atropine: effects in HEOD-treated adult rats..... | 130 |
| K. | L- α -methyldopa: effects in HEOD-treated adult rats..... | 137 |
| L. | d-1-Propranolol: effects in HEOD-treated adult rats..... | 144 |
| M. | Summary of results..... | 149 |
| Mi. | Acute toxicity of dieldrin in the three age groups..... | 149 |

| | | |
|-------|--|-----|
| Mii. | Effects of dieldrin on carbohydrate metabolism in five and ten day olds..... | 149 |
| Miii. | Effects of dieldrin on carbohydrate metabolism in the adult male rat..... | 150 |

DISCUSSION

| | | |
|-------|--|-----|
| A. | Acute toxicity of HEOD administered <u>per os</u> | 154 |
| B. | Effects of d-glucose on the acute toxicity of HEOD in the immature rat..... | 155 |
| C. | Carbohydrate metabolism in the immature rat - Effects of HEOD..... | 168 |
| D. | Toxic versus adaptive nature of the HEOD-induced hyperglycemic response in the adult rat..... | 173 |
| E. | Etiology of the HEOD-induced carbohydrate response in the adult rat..... | 179 |
| Ei. | The literature..... | 179 |
| Eii. | A new model to explain the elicitation and control of the HEOD-induced hyperglycemic response..... | 182 |
| Eiii. | The model..... | 198 |
| Eiv. | Experimental support for the model..... | 195 |
| Ev. | Alternative explanations..... | 200 |
| F. | Conclusions..... | 202 |
| Fi. | Immature animals..... | 202 |
| Fii. | Mature animals..... | 203 |
| Fiii. | Animals of all ages..... | 203 |

| | |
|-----------------------|-----|
| LITERATURE CITED..... | 204 |
|-----------------------|-----|

| | |
|-----------------|-----|
| APPENDICES..... | 219 |
|-----------------|-----|

| | |
|--------------------|-----|
| VITA AUCTORIS..... | 223 |
|--------------------|-----|

LIST OF TABLES

TABLE

page

| | | |
|-------|--|----|
| I. | Age-specific dieldrin LD50 values (per os) in five and ten day old pups (mixed sexes) and in 60 day old adult males..... | 71 |
| II. | Plasma glucose, plasma FFA and hepatic glycogen in five day old pups, one and three hours after 1 LD50 HEOD..... | 72 |
| III. | Plasma glucose, plasma FFA and hepatic glycogen in ten day old pups, one and three hours after 1/2 LD50 HEOD..... | 74 |
| IV. | Plasma glucose, plasma FFA and hepatic glycogen in ten day old pups, one and three hours after 1 LD50 HEOD..... | 75 |
| V. | Plasma glucose, plasma FFA and hepatic glycogen in 60 day old male rats, one and three hours after 1 LD50 HEOD..... | 77 |
| VI. | Percent increase in treated versus control mean plasma glucose concentrations found for: 60, 120 and 200 day old male rats at various times after 1/2 or 1, 60 day old age-specific LD50 HEOD (i.e. 31.5 or 63.0 mg/kg)..... | 78 |
| VII. | Plasma glucose, plasma FFA and hepatic glycogen in 120 and 200 day old male rats, one or one and one-half hours after HEOD (dose: 1/2 or 1, 60 day old age-specific LD50 - i.e. 31.5 or 63.0 mg/kg)..... | 80 |
| VIII. | Plasma glucose concentrations (mg/dl) in five and ten day old pups (mixed sexes) and 60 day old male rats, one and three hours after HEOD (1 age-specific LD50)..... | 81 |
| IX. | Plasma glucose concentration (mg/dl) in control animals of substrate and enzyme studies. Data was collected for five and ten day old pups (mixed sexes) and 60 day old male rats, one and three hours after corn oil vehicle..... | 83 |
| X. | Plasma glucose concentration (mg/dl) in HEOD-treated animals of substrate and enzyme studies. Data was collected for five and ten day old pups (mixed sexes) and 60 day old male rats, one and three hours after HEOD (1 age-specific LD50)..... | 84 |

- XI. Hepatic fructose-1,6-diphosphatase activity (U/g wet liver weight) in five and ten day old pups (mixed sexes) and 60 day old male rats, one and three hours after HEOD (1 age specific LD50).....85
- XII. Hepatic glucose-6-phosphatase activity (U/g wet liver weight) in five and ten day old pups (mixed sexes) and 60 day old male rats, one and three hours after HEOD (1 age-specific LD50).....87
- XIII. Hepatic phosphoenolpyruvate carboxykinase activity (U/g wet liver weight) in five and ten day old pups and 60 day old male rats, one and three hours after HEOD (1 age-specific LD50).....88
- XIV. Results of 2-way anova on mean plasma glucose concentrations observed in 60 day old male rats given HEOD (1 LD50) and d-glucose (6 g/kg p.o.) every two hours for 24 hours.....91
- XV. Mortality at 24 and 48 hours in ten day old pups (mixed sexes) treated with 1 LD50 HEOD and either, 6 g/kg p.o. d-glucose (treated) or distilled water (control) at: 0, 3, 6 and 18 hours after HEOD.....95
- XVI. Plasma glucose concentrations (mg/dl) in 60 day old male rats given 1 LD50 HEOD and either, 2 U/kg s.c. semi-lente insulin (treated) or insulin diluent (control).....100
- XVII. Convulsion latency time (CLT), 24 hour mortality and plasma glucose, hepatic glycogen and PEPCK activity, two hours post-treatment in 60 day old male rats given 1 LD50 HEOD and either, 2 U/kg s.c. semi-lente insulin (treated) or insulin diluent (control)..101
- XVIII. Plasma glucose concentrations (mg/dl) in 60 day old male rats given phenobarbital (40 mg/kg i.p.) and either, 1 LD50 HEOD (treated) or corn oil (control).....106
- XIX. Convulsive latency time (CLT), 24 hour mortality and hepatic glycogen and PEPCK activity, two hours post-treatment in 60 day old male rats given phenobarbital (40 mg/kg i.p.) and either, 1 LD50 HEOD (treated) or corn oil (control).....108

- XX. Convulsion latency time (CLT), 24 hour mortality and hepatic glycogen and PEPCK activity, two hours post-treatment in 60 day old male rats given phenobarbital (40 mg/kg i.p.) exogenous glucose (6 g/kg p.q. at 0, 3, 9 and 18 hours after phenobarbital) and either, 1 LD50 HEOD (treated) or corn oil (control)112
- XXI. Convulsion latency time (CLT), 24 hour mortality and hepatic glycogen and PEPCK activity, two hours post-treatment in 60 day old male rats given phenobarbital (40 mg/kg i.p.) semi-lente insulin (2 U/kg s.c.) and either, 1 LD50 HEOD (treated) or corn oil (control).....117
- XXII. Results of 2-way anova on mean plasma glucose concentrations (mg/dl) during first six hours of experimentation in 60 day old male rats treated with either, 1 LD50 HEOD and 2 U/kg s.c. semi-lente insulin (data of treated group - Table XVI) or HEOD, insulin and 40 mg/kg i.p. phenobarbital.....119
- XXIII. Data comparison - convulsion latency time (CLT), 24 hour mortality and hepatic glycogen and PEPCK activity, two hours after treatment with either, 1 LD50 HEOD and 2 U/kg s.c. semi-lente insulin (data of treated group - Table XVII) or HEOD, insulin and 40 mg/kg i.p. phenobarbital (data of treated group - Table XXI).....120
- XXIV. Derivation of corrected mean plasma glucose (i.e. "glucose - 2-deoxyglucose") in 60 day old male rats given 1 LD50 HEOD and either, 1 g/kg s.c. 2-deoxyglucose (treated) or distilled water (control) and results of 2-way paired anova on corrected plasma glucose of treated rats versus plasma glucose of control rats.....127
- XXV. Convulsion latency time (CLT) and 24 hour mortality in 60 day old male rats given 1 LD50 HEOD and either, 1 g/kg s.c. 2-deoxyglucose (treated) or distilled water (control).....128
- XXVI. Mortality in 60 day old male rats given 1 LD50 HEOD and either, 1 g/kg s.c. 2-deoxyglucose (treated) or distilled water (control).....129
- XXVII. Convulsion latency time (CLT), 24 hour mortality and hepatic glycogen and PEPCK activity, two hours post-treatment in 60 day old male rats given 1 LD50 HEOD and either, 4 mg/kg s.c. atropine (treated) or 0.9% saline (control) at: 40 minutes prior to, and 4, 8, 12 and 18 hours after HEOD.....134

- XXVIII. Mortality in 60 day old male rats given 1 LD50 HEOD and either, 4 mg/kg s.c. atropine (treated) of 0.9% saline (control) at: 40 minutes prior to, and 4, 8, 12 and 18 hours after HEOD.....135
- XXIX. Convulsion latency time (CLT), 24 hour mortality and hepatic glycogen, G6P and PEPCK activity, two hours post-treatment in 60 day old male rats given 1 LD50 HEOD and either, 200 mg/kg i.p. L-methyldopa (treated) or acid-saline, (control) at 0 and 6 hours after HEOD.....141
- XXX. Convulsion latency time (CLT), 24 hour mortality and hepatic glycogen and PEPCK activity, two hours post-treatment in 60 day old male rats given 1 LD50 HEOD and either, 8 mg/kg s.c. propranolol (treated) or 0.9% saline (control) at 15 minutes prior to, and 5 and 3/4 hours after HEOD.....148
- XXXI. Summary of effects produced on HEOD-induced hyperglycemia, CNS hyperactivity (convulsion latency time) and mortality (24 hour) in experiments performed on 60 day old male rats.....153

LIST OF FIGURES

| <u>FIGURE</u> | <u>page</u> |
|---|-------------|
| 1. Phosphoenolpyruvate carboxykinase assay, enzyme kinetics - NADH oxidized ($\mu\text{mole} \times 10^{-2}/\text{ml}$ of assay mix) versus time (minutes). Curve represents mean of 10 samples..... | 55 |
| 2. Phosphoenolpyruvate carboxykinase assay, enzyme kinetics - NADH oxidized ($\mu\text{mole} \times 10^{-3}/\text{ml}$ of assay mix versus enzyme concentration (microsomal protein mg/ml in assay mix). Curve represents mean of five replicates..... | 58 |
| 3. Phosphoenolpyruvate carboxykinase assay, substrate saturation - NADH oxidized ($\mu\text{mole} \times 10^{-3}/\text{ml}$ of assay mix/minute) versus substrate concentration (phosphoenolpyruvate, PEP, mM). Curve represents mean of five samples..... | 60 |
| 4. Mean plasma glucose (mg/dl) in 60 day old male rats given either 1 LD50 HEOD (treated) or corn oil (control) and d-glucose (6 g/kg p.o.) every two hours for 24 hours..... | 92 |
| 5. Mean plasma glucose (mg/dl) in 60 day old male rats given 1 LD50 HEOD and either 2 U/kg s.c. semi-lente insulin (treated) or insulin diluent (control)..... | 98 |
| 6. Mean plasma glucose (mg/dl) in 60 day old male rats given 40 mg/kg i.p. sodium phenobarbital and either 1 LD50 HEOD (treated) or corn oil (control)..... | 104 |
| 7. Mean plasma glucose (mg/dl) in 60 day old male rats given 40 mg/kg i.p. sodium phenobarbital and at the times indicated 6 g/kg p.o. d-glucose and either 1 LD50 HEOD (treated) or corn oil (control)..... | 110 |
| 8. Mean plasma glucose (mg/dl) in 60 day old male rats given 40 mg/kg i.p. sodium phenobarbital, 2 U/kg s.c. semi-lente insulin and either 1 LD50 HEOD (treated) or corn oil (control)..... | 115 |
| 9. Mean plasma glucose (mg/dl) in 60 day old male rats given 1 LD50 HEOD and either 1 g/kg s.c. 2-deoxy-glucose (treated) or distilled water (control)..... | 124 |

10. Mean plasma glucose (mg/dl) in 60 day old male rats given 1 LD50 HEOD and either 4 mg/kg s.c. atropine (treated) or 0.9% saline (control).....131
11. Mean plasma glucose (mg/dl) in 60 day old male rats given 1 LD50 HEOD and either 200 mg/kg i.p. L- α -methyldopa (treated) or acid-saline (control).....138
12. Mean plasma glucose (mg/dl) in 60 day old male rats given 1 LD50 HEOD and either 8 mg/kg s.c. d,l-propranolol (treated) or 0.9% saline (control).....146
13. Possible pathways utilized in carbohydrate metabolism in the fasted, HEOD-treated immature rat.....174
14. Diagramatic representation of the model proposed to explain the etiology of the HEOD-induced hyperglycemic response in the adult rat.....191

LIST OF APPENDICES

| <u>Appendix</u> | <u>page</u> |
|---|-------------|
| 1. List of chemicals and suppliers..... | 219 |
| A. Chemicals (by assay or preparation)..... | 219 |
| Ai. 2-deoxyglucose (plasma) assay..... | 219 |
| Aii. Free fatty acid (plasma) assay..... | 219 |
| Aiii. Fructose-1,6-diphosphatase (hepatic) assay..... | 219 |
| Aiv. Glucose (plasma) assay..... | 219 |
| Av. Glucose-6-phosphatase (hepatic) assay..... | 220 |
| Avi. Glycogen (hepatic) assay..... | 220 |
| Avii. Phosphosphoenolpyruvate carboxykinase (hepatic) assay..... | 220 |
| Aviii. Profein - Lowry (hepatic) assay..... | 221 |
| B. Diluent Preparations..... | 221 |
| Bi. Insulin diluent composition..... | 221 |
| Bii. Phenobarbital diluent composition..... | 221 |
| C. Drug suppliers..... | 221 |
| D. Supplier addresses..... | 221 |
| 2. Method of preparation of diluent for semi-lente insulin..... | 222 |

INTRODUCTION

The chlorinated hydrocarbon insecticides (DDT, dieldrin, chlordane etc.) produce dose-dependant perturbations in the in vivo carbohydrate metabolism of the rat (Hiddemen & Cornish, 1970; Singhal et al., 1970; Bhatia et al., 1972, 1973; Kacew et al., 1972a, 1972b,; Kacew & Singhal, 1973a, 1973b, 1973c, 1973d; Hickenbottom & Yau, 1974; Kohli et al., 1975b) rabbit, (Stohlman & Lillie, 1948) rhesus monkey (Dudeja et al., 1980) and Indian catfish (Srivastava & Singh, 1981). Similarly, these agents alter gluconeogenesis in vitro in rat hepatic (Kacew & Singhal, 1973c, 1974a, 1974b; Story & Freedland, 1978a, 1978b, 1979) and renal tissue (Kacew et al., 1972a, 1972b; Kacew & Singhal, 1973c, 1973d, 1974a, 1974b).

Hyperglycemia is the most pronounced effect in vivo (Stohlman & Lillie, 1948; Hiddemen & Cornish, 1970; Bhatia et al., 1972; Kacew et al., 1972a, 1972b; Kacew & Singhal, 1973a, 1973b, 1973c). This hyperglycemia results in peak plasma glucose concentrations within one to five hours after administration of an agent to the adult rat (Kacew et al., 1972a) and rabbit (Stohlman & Lillie, 1948). This effect may persist for up to 24 hours following insecticide exposure in the rat (Hiddemen & Cornish, 1970; Bhatia et al., 1972).

The hyperglycemia is believed to result from enhanced gluconeogenesis since the activities of hepatic phosphoenolpyruvate carboxykinase, pyruvate carboxylase, fructose-1,6-diphosphatase and glucose-6-phosphatase are all increased

following exposure to organochlorine insecticides (Singhal et al., 1970; Kacew et al., 1972a, 1972b; Kacew & Singhal, 1973a, 1973b, 1973c, 1973d; Bhatia et al., 1973; Hickenbottom & Yau, 1974; Kohli et al., 1975b; Bandyopadhyay et al., 1982a).

Glycogen metabolism is also altered in vivo in the rat (Singhal et al., 1970; Bhatia & Venkitasubramanian, 1972; Kacew et al., 1972b; Kacew & Singhal, 1973a, 1973b, 1973d; Bhatia et al., 1973; Hickenbottom & Yau, 1974) rhesus monkey, (Agarwal et al., 1978; Dudeja et al., 1980) and Indian catfish (Srivastava & Singh, 1981). In the rat enhanced glycogenolysis and inhibition of glycogenesis occurs within one hour of administration of an agent (Kacew & Singhal, 1973a, 1973b, 1973d). The longer term effects (i.e. 18 - 24 hours) are not clear. Bhatia et al. (1973) reported increased hepatic glycogen content and elevated glycogen synthetase activity, 24 hours after exposure to dieldrin. Conversely, decreased hepatic glycogen content and enhanced glycogen phosphorylase activity were found 18 hours after treatment with DDT (Hickenbottom & Yau, 1974). Thus in the adult mammal, the organochlorine insecticides readily induce a "carbohydrate response" characterized by hyperglycemia and enhanced hepatic gluconeogenesis and glycogenolysis.

The effect of organochlorine insecticides on carbohydrate metabolism in the immature mammal has not been extensively studied. Costella & Virgo (1980) studied the effect of in utero exposure to dieldrin on neonatal carbohydrate metabolism. These authors found that the congenital inviability produced

in mice by such exposure was associated with perturbed carbohydrate metabolism. The inviable pups exhibited enhanced post-partum hypoglycemia and hepatic glycogenolysis.

Gluconeogenesis, in contrast to the situation described above for the adult rodent, was found to be essentially normal in these inviable pups. This suggests that the effect of the organochlorine insecticides on carbohydrate metabolism is different in the immature and the mature mammal.

The etiology of the response is still unknown, despite several studies. The most comprehensive model presented to date is that of Kacew & Singhal (1974a, 1974b). This model suggests that organochlorine insecticides stimulate gluconeogenesis by binding to and stimulating the activity of adenyl cyclase. This model and the results on which it is based, have yet to be confirmed by other investigators.

In fact, the current evidence indicates the organochlorine insecticides do not activate adenyl cyclase. Thus, Poschl et al. (1982) have reported no change in the adenyl cyclase activity of hepatocytes obtained from DDT-exposed rats and Dudeja et al. (1980) found that hepatic adenyl cyclase activity was actually decreased in the rhesus monkey treated with DDT.

There is also some question as to the physiological importance of organochlorine-enhanced hepatic adenyl cyclase activity, since Story & Freedland (1978a, 1979) reported that gluconeogenesis in hepatocytes obtained from adult rats chronically fed DDT, exhibited a decreased sensitivity to cAMP.

stimulation.

To date, there has been no investigation of the possible contribution of the organochlorine-induced carbohydrate response to the acute toxicity of any of the insecticides. These compounds are all primary neurotoxins (O'Brien, 1967; Murphy, 1980) and the mammalian central nervous system (CNS) is glucose dependant (Ensinck & Williams, 1981). The onset of insecticide-induced hyperglycemia correlates well with the period of insecticide-induced convulsions and death (Stohlman & Lillie, 1948; Kacew et al., 1972a, 1972b). Thus, the insecticide-induced carbohydrate response of the mammal may be a component in the lethal action of these agents.

In consideration of the above studies, this present study was undertaken with the following specific objectives in view:

- 1) To characterize the effect of the cyclodiene insecticide, dieldrin, on carbohydrate metabolism in the immature and the mature rat.
- 2) To determine the significance of the dieldrin-induced carbohydrate response in relation to the acute toxicity of the agent in the immature and the mature rat.
- 3) To determine the physiological mechanism(s) by which dieldrin perturbs carbohydrate metabolism in the rat.

LITERATURE REVIEW

Chlorinated hydrocarbon insecticides - Effects on carbohydrate metabolism.

A. The adult rat.

Stohlman & Lillie (1948) first reported the ability of DDT (dichloro-diphenyl-trichloroethane) to cause hyperglycemia in the rabbit. These authors found that a single dose of DDT (28 mg/kg i.v.) elevated blood glucose concentration by 100 to 200% within one to two hours. When the pesticide was given *per os*, dissolved in corn oil (dose 600 mg/kg), a maximal increase of 35% in mean blood glucose occurred seven hours later. The authors also found a decreased rectal temperature in these animals. However, this effect was thought to be caused by the corn oil and not the DDT.

Stohlman & Lillie (1948) also examined the effect that administering glucose (1 g/kg i.v. as a 10% solution in water) at 8, 18, 42 and 66 hours had on blood glucose concentration in the rabbit given 600 mg/kg DDT *p.o.* dissolved in corn oil. This exogenous glucose did not alter mean blood glucose concentration in DDT-treated rabbits at 18, 42 or 66 hours. Since the authors had already established that the hyperglycemic effect of DDT was transitory (i.e., producing peak hyperglycemia at about seven hours after pesticide exposure and normal levels by 18 hours), it is perhaps surprising that they did not examine the effects of exogenous glucose during the early period of DDT-induced hyperglycemia. The mortality observed over a

seven day period following exogenous glucose and DDT was 38%, while that seen over the same period in rabbits given an equivalent dose of DDT alone was 58%. Therefore, exogenous glucose treatment did not alter the acute toxicity of DDT.

The authors also determined the extent of DDT-induced pathological changes in rabbits given, for ten days, either DDT (158 mg/kg/day p.o.) or DDT and exogenous glucose (1-5 g/kg/day i.v. as a 10% solution). At the end of the ten day period, treatments were discontinued and the rabbits were necropsied subsequent to their deaths. Stohlman and Lillie's paper makes no reference as to when their animals died other than to state that mean survival in the treated group (i.e. DDT plus glucose) was 27.3 days versus 45.8 days in the untreated group (i.e. DDT alone). It is assumed from this statement that rabbits were necropsied whenever they died and that no attempt was made to examine the extent of pathological changes produced by DDT in either group at some standard time following chronic treatment.

The authors examined the extent of necrosis and "fatty degenerative changes" seen in the liver, kidney, heart, lung, pancreas and adrenal gland. Both groups invariably exhibited "fatty livers". However, hepatic necrosis was seen in only 48% of the control (non-glucose exposed) animals versus 77% of the treated (glucose-exposed) rabbits. Levels of hepatic glycogen (presumably at the time of necropsy) were found to be "small to moderate" in 88% of the glucose-treated animals. Conversely, 94% of the non-glucose treated animals had detectable levels of

hepatic glycogen. Fatty degeneration of kidney tubules was seen in 50% of the non-glucose treated rabbits while 95% of treated rabbits exhibited this condition. Congestion and edema of the lung was seen in 66% of the untreated animals and in 50% of treated rabbits. Fatty degeneration of heart muscle was observed in 95% of untreated animals and in 40% of the glucose-treated rabbits. The pancreas and adrenals were studied in only some (no numbers stated) of the animals. The authors stated that no lesions of any significance occurred in these organs in either group. The final conclusion reached by the authors was that "the glucose treatment apparently decreased the extent and frequency of lesions in the organs studied. This apparent decrease in lesions however, was not associated with a longer survival time or increased tolerance to the poison" (Stohlman & Lillie, 1948).

One of the earliest reports of altered hepatic enzyme activities after DDT is that of Platt and Cockrell (1969). These authors fed pp-DDT (0.10% w/w in the diet) to rats for 14 days. DDT increased the following parameters in liver: weight (86%); microsomal protein concentration (29%); and the activities of NADPH₂-cytochrome c reductase (261%), aminopyrine demethylase (128%) and b-phosphogluconate dehydrogenase (38%) when compared to non-DDT treated rats. The pesticide also decreased the activities of hepatic G6P (31.5%), lactic acid dehydrogenase (43%) and glutamate dehydrogenase (47%).

The authors concluded that chronic administration of DDT to the rat stimulated microsomal oxidative drug metabolism and

increased microsomal protein concentration equivalent to that produced by a barbiturate while also marginally reducing the activity of some hepatic enzymes such as glucose-6-phosphatase.

As an aside, increased hepatic microsomal protein concentration is not a prerequisite for enhanced hepatic drug-metabolizing enzyme activity following DDT exposure. This was recently shown by Pasha (1981), who fed 280 ppm DDT in the diet to CF-1 mice for seven days and found elevated activities of hepatic cytochrome P450 and cytochrome-c reductase in the face of unchanged hepatic microsomal protein concentration.

The effect of DDT on the activities of several enzymes involved in carbohydrate metabolism in rat uterine tissue was studied by Singhal et al. (1970). This study found that a single dose of op-DDT (10 mg/100g i.v.) administered to ovariectomized rats caused significant increases in: uterine weight (75%); glycogen content (285%); and the activities of hexokinase (169%), phosphofructokinase (133%), aldolase (173%), pyruvate kinase (163%), glucose-6-phosphate dehydrogenase (285%) and 6-phosphogluconate dehydrogenase (97%). The increased uterine glycogen content was only elicited by the op-DDT isomer.

All of the above effects of op-DDT were inhibited in ovariectomized rats given either actinomycin D (25 µg/100g) or cycloheximide (70 µg/100g). Inhibition of DDT-induced effects could also be achieved by treating rats with progesterone (5 mg/100g) 30 minutes prior to op-DDT. On the other hand, estradiol (0.1 µg/100g) potentiated the effects of op-DDT on

uterine tissue. It was concluded that DDT possesses uterotropic activity and acts in an analogous fashion to estrogens to induce the de novo synthesis of several uterine enzymes involved in carbohydrate metabolism.

Also in 1970, the first report of dieldrin-induced hyperglycemia in rats appeared. Hiddemen and Cornish (1970) treated adult male Sprague Dawley rats with 95% HEOD dissolved in corn oil (dose 25 mg/kg/day i.p.) for 1, 2, 3, and 4 days respectively.

Following HEOD treatment, the rats were fasted for 21 hours and then killed for analysis of blood glucose and plasma corticosterone. The four treatment groups were found to have blood glucose concentrations 18, 24, 47, and 60% greater than those levels found in corn oil-treated controls. Dieldrin-treated rats also exhibited elevated plasma corticosterone concentrations of 45, 102, 97 and 98% respectively. Finally, adrenalectomized rats, given HEOD as described above, failed to produce any hyperglycemic response. The authors concluded that induced hyperglycemia in the rat results as a consequence of a HEOD-induced release of corticosterone from the adrenal cortex.

Enhanced adrenal corticoid activity in the HEOD-treated rat was also inferred in the report of Bhatia et al. (1971). These authors gave male Wistar rats (110-125 g) a single dose of HEOD (30 mg/kg p.o. dissolved in ground-nut oil). Next, the rats were subjected to a 24 hour fast, then killed and the ascorbic acid content of plasma, liver, kidney, spleen,

heart and brain was assessed. Additionally, the weights and ascorbic acid/cholesterol content of their adrenals were measured. Dieldrin increased the ascorbic acid content of plasma, liver, kidney, spleen, heart and brain. The pesticide did not alter mean adrenal weight. However, it did significantly reduce adrenal ascorbic acid and cholesterol content.

According to Bhatia et al. (1971) cholesterol acts as a precursor for adrenal steroidogenesis. Thus, depleted adrenal ascorbic acid levels are associated with enhanced adrenal secretory function. Consequently, the authors concluded that the observed reduced adrenal concentrations of these two parameters was indicative of HEOD-enhanced adrenal cortical activity in the rat.

Bhatia and Venkitasubramanian (1972) also studied the "fatty liver" effect induced by dieldrin. These authors examined lipid metabolism in male Wistar rats given HEOD (30 mg/kg p.o.) and killed after a subsequent 24 hour fast. Dieldrin increased hepatic weight, total lipid and triglyceride content. It also increased liver glycogen by 200%. HEOD did not alter liver protein, phospholipid or cholesterol levels. Plasma triglyceride, phospholipid and free fatty acid (FFA) levels were all increased by HEOD.

The incorporation of [^{14}C]-glucose into hepatic lipids of HEOD-treated rats was also studied. The animals received HEOD (30 mg/kg p.o.) and were then fasted for 20-22 hours prior to being given [^{14}C]-glucose (specific activity: 30 $\mu\text{Ci}/200\text{mg}$; dose: 30 $\mu\text{Ci}/100\text{g}$ body weight). Three hours after receiving

the radioactive glucose, the rats were killed and the following results were found. Dieldrin pretreatment decreased the activity of the hepatic fatty acid synthesizing enzyme system (as evidenced by a reduced rate of in vitro incorporation of [^{14}C]-labelled acetate into fatty acids). Also, the incorporation of [^{14}C]-glucose into total hepatic fatty acids was decreased.

Conversely, [^{14}C]-glucose incorporation into hepatic total lipid, neutral glycerides (i.e. triglycerides) and glyceride-glycerol were all increased by HEOD. The pesticide did not alter [^{14}C]-glucose incorporation into either hepatic cholesterol or phospholipids. Thus, the "fatty liver" seen in HEOD-treated rats resulted from decreased hepatic esterification of available FFA and glycerophosphate (recall that HEOD treatment produced an enhanced pool of these precursors). However, HEOD inhibited hepatic lipogenesis (as evidenced by a decreased [^{14}C]-glucose incorporation into fatty acids and a reduced activity of the hepatic fatty acid synthesizing enzyme system). Therefore, the fatty liver effect of dieldrin was attributed to an increased esterification of fatty acids mobilized by the pesticide from adipose tissue.

The results of Bhatia and Venkatasubramanian (1972) were confirmed in 1975 in a study performed by Kohli et al. (1975a). These authors gave adult male Wistar rats HEOD (38 mg/kg p.o.) followed by a 24 hour fast. Three hours prior to the end of that fast, the rats were injected with [^{14}C]-acetate (specific activity: 46.8 mCi/mole; dose: 15 $\mu\text{Ci}/100\text{g}$

body weight).

As Bhatia and Venkitasubramanian (1972) had previously found, Kohli et al. (1975a) also observed increased hepatic weight, total lipid, fatty acid and triglyceride content, and unchanged hepatic cholesterol and phospholipid levels after HEOD treatment. Additionally, the incorporation rate of [14 C]-acetate into hepatic fatty acids and triglycerides was decreased.

However, when [14 C]-palmitate was added to hepatic incubates taken from animals given only HEOD, the rate of [14 C]-incorporation into respiratory CO_2 and hepatic triglyceride was significantly increased. Thus, it was concluded that HEOD had increased the rate of hepatic fatty acid oxidation and inhibited de novo fatty acid synthesis. Finally, Kohli et al. (1975a) also believed that the increased hepatic triglyceride concentration resulted from HEOD-enhanced esterification of FFA mobilized from adipose tissue.

The most recent study regarding the effects of an organochlorine insecticide on lipid metabolism in the adult rat is that of Rao et al. (1981). These workers gave a single dose of pp-DDT (600 mg/kg p.o.) to male Wistar rats which were killed five hours later. Neither plasma nor adipose tissue total lipid content, triglyceride or cholesterol concentrations were changed by DDT. Furthermore, DDT did not alter either liver or adipose tissue lipoprotein lipase activity. However, DDT decreased hepatic phospholipid and triglyceride concentrations and decreased plasma lipoprotein lipase activity.

Rao et al. (1981) concluded that DDT had no effect on

lipid metabolism in adipose tissue and had only a minor effect on lipid metabolism in hepatic tissue. In view of this study, it seems probable that the effects on lipid metabolism reported by Bhatia and Venkitasubramanian (1972) and Kohli et al. (1975a) only occur late after pesticide exposure.

Bhatia et al. (1972) studied the effects of technical grade dieldrin (85% HEOD and 15% other compounds) on several parameters of carbohydrate metabolism in the rat. Adult Wistar rats were given technical HEOD (30 mg/kg p.o.) and subjected to a 24 hour fast. After fasting, the animals were killed and the concentrations of glucose, FFA, pyruvic and lactic acid in blood were measured. In addition, hepatic lactic acid concentration was assayed. In other experiments, glucose tolerance tests were performed. The authors found that all blood and liver parameters were significantly increased in dieldrin-exposed animals while glucose tolerance was decreased. This latter result is particularly interesting since it indicates that HEOD impairs the uptake and utilization of glucose.

Bhatia et al. (1972) explained their results on the basis of an observed HEOD-induced elevation of plasma corticosterone. Adrenal corticoids are known to depress glucose tolerance and to stimulate lipolysis and gluconeogenesis. The increased concentrations of blood pyruvic acid and hepatic and blood lactic acid were thought to result from a FFA-induced impairment of pyruvate oxidation in the HEOD-treated rat.

Also in 1972, Kacew et al. (1972a) studied the stimulatory effect of DDT on the activities of renal and hepatic glucone-

genic enzymes. These authors found that pp'DDT (600 mg/kg p.o.) given to 16 hour starved adult Wistar rats increased the activity of renal phosphoenolpyruvate carboxykinase (PEPCK), pyruvate carboxylase (PC), fructose-1,6-diphosphatase (FDP) and glucose-6-phosphatase (G6P).

The enhanced renal enzyme activities were shown to be both dose-dependant (100 mg/kg pp'DDT representing a minimum effective oral dose) and time-dependant (maximal induction occurring about five hours after pesticide exposure). Hepatic gluconeogenic enzyme activities were similarly enhanced (i.e. a two fold increase) five hours after pp'DDT (600 mg/kg p.o.). Furthermore, the stimulatory effect of pp'DDT on renal gluconeogenic enzymes was not mediated by adrenocorticoid hormones, since adrenalectomy did not inhibit the effect. Additionally, the effect was not augmented in adrenalectomized rats given the glucocorticoid, triamcinolone (10 mg/100 g).

Kacew et al. (1972a) also found that pretreatment with RNA or protein synthesis inhibitors (actinomycin D, cycloheximide or ethionine) blocked pp'DDT-increased gluconeogenic enzyme activity. However, if these inhibitors were administered in conjunction with pp'DDT, then no inhibition of enhanced gluconeogenic enzyme activity occurred. Both op'DDT and pp'DDT were equally effective in enhancing renal enzyme activity. Furthermore, this DDT effect was not sex-specific. Finally, the authors demonstrated that chronic exposure to a small dose of pp'DDT (0.5 mg/100g/day p.o. for 45 days) also stimulated renal and hepatic gluconeogenic enzyme activities.

During 1972 and 1973, Kacew in collaboration with various authors published several papers dealing with the organo-chlorine-induced carbohydrate response of the adult rat.

Rather than review each of these papers individually, a summary of the pertinent results will be presented.

Thus:

a) Acute administration of the following insecticides increased renal and hepatic PEPCK, G6P, FDP and PC activities:

op' DDT - (600 mg/kg : Kacew et al., 1972a)
pp' DDT - (600 mg/kg : Kacew et al., 1972a;
Kacew & Singhal, 1973a, 1973b)
chlordan - (200 mg/kg : Kacew & Singhal, 1973b)
heptachlor - (200 mg/kg : Kacew & Singhal, 1973b)
endrin - (50 mg/kg : Kacew & Singhal, 1973b)

b) Hyperglycemia, hyperuremia and decreased hepatic glycogen content followed administration of:

pp'DDT - (600 mg/kg : Kacew & Singhal, 1973a, 1973b)
 chlordane - (200 mg/kg : Kacew & Singhal, 1973b)
 heptachlor - (200 mg/kg : Kacew & Singhal, 1973b)
 endrin - (50 mg/kg : Kacew & Singhal, 1973b)

c) A 688 mg/kg dose of pp'DDT caused:

i) elevated gluconeogenic enzyme activities (renal) in adrenalectomized rats (Kacaw & Singhal, 1972a)

- ii) no augmentation in elevated gluconeogenic enzyme activity in adrenalectomized rats given triamcinolone (10 mg/kg)
 - renal - (Kacew et al., 1972a)
 - renal and hepatic - (Kacew & Singhal, 1973a)

- iii) hyperglycemia, and decreased hepatic glycogen content in adrenalectomized rats (Kacew & Singhal, 1973a)

iv) increased gluconeogenic enzyme activity with concurrent treatment with RNA/protein synthesis inhibitors - (Kacew et al., 1972a; Kacew & Singhal, 1973a)

d) Finally, the minimal effective acute dose of pp-DDT required to enhance gluconeogenic enzyme activity was 100 mg/kg - (Kacew et al., 1972a; Kacew & Singhal, 1973a).

In regards to the induced carbohydrate response elicited by chronic administration of organochlorine pesticides, the following results were found:

a) Minimum effective chronic doses to elevate activities of renal gluconeogenic enzymes were:

op'DDT - (10 mg/kg/day, 3 days : Kacew et al., 1972b)
 pp'DDT - (5 mg/kg/day, 45 days : Kacew & Singhal, 1973a)
 heptachlor - (3 mg/kg/day, 45 days : Kacew et al., 1973)
 heptachlor epoxide - (5 mg/kg/day, 45 days :

Kacew et al., 1973)

endrin - (2 mg/kg/day, 45 days : Kacew et al., 1973)

chlordan - (5 mg/kg/day, 20 days : Kacew & Singhal, 1973d)

b) Minimum effective chronic doses to elevate activities of hepatic gluconeogenic enzymes were:

op'DDT - (10 mg/100g/day, 7 days : Kacew et al., 1972b)

pp'DDT - (5 mg/kg/day, 45 days : Kacew et al., 1972a;
 Kacew & Singhal, 1973a)

heptachlor - (15 mg/kg/day, 45 days : Kacew et al., 1973)

heptachlor epoxide - (5 mg/kg/day, 45 days :

Kacew et al., 1973)

chlordan - (25 mg/kg/day, 45 days : Kacew & Singhal, 1973d)

endrin - (0.5 mg/kg/day, 45 days : Kacew et al., 1973)

c) Hyperglycemia developed after 45 days with:

heptachlor - (3 mg/kg/day : Kacew et al., 1973)

heptachlor epoxide - (1 mg/kg/day : Kacew et al., 1973)

endrin - (0.5 mg/kg/day : Kacew et al., 1973)

d) Hepatic glycogen content was decreased after 45 days with:

heptachlor - (15 mg/kg/day : Kacew et al., 1973)

heptachlor epoxide - (5 mg/kg/day : Kacew et al., 1973)

endrin - (2 mg/kg/day : Kacew et al., 1973)

e) Hyperuremia developed after 45 days with:

chlordan - (5 mg/kg/day : Kacew & Singhal, 1973d)

heptachlor - (3 mg/kg/day : Kacew et al., 1973)

heptachlor epoxide - (1 mg/kg/day : Kacew et al., 1973)

endrin - (0.5 mg/kg/day : Kacew et al., 1973)

f) Glycosuria followed chronic treatment with:

op'DDT - (10 mg/kg/day, 7 days : Kacew et al., 1972b)

pp'DDT - (25 mg/kg/day, 20 days : Kacew & Singhal, 1973a)

g) Triamcinolone (1 mg/100g/day for 3 days) did not further augment the enhanced renal gluconeogenic enzyme activity

produced by chronic exposure to op-DDT (10mg/100g/day for 3 days): (Kacew et al., 1972b)

h) Decreased liver weight followed 45 day treatment with:

heptachlor - (3 mg/kg/day : Kacew et al., 1973)
 heptachlor epoxide - (1 mg/kg/day : Kacew et al., 1973)
 endrin - (0.5 mg/kg/day : Kacew et al., 1973)

i) Decreased body and kidney weights developed after administering endrin (0.5 mg/kg/day for 45 days :
 Kacew et al., 1973)

j) Testicular weight decreased in rats given heptachlor (15 mg/kg/day for 45 days : Kacew et al., 1972b)

Bhatia et al. (1973) extensively studied the effects of dieldrin on carbohydrate metabolism in adult male Wistar rats. The animals were given HEOD (30 mg/kg p.o.) and were then fasted for 24 hours prior to being killed. Dieldrin exposure increased liver weight, protein and RNA concentrations. It also caused a four-fold increase in hepatic glycogen content and increased the incorporation of [14 C]-glucose, [14 C]-glycine and [14 C]-bicarbonate into liver glycogen. Dieldrin enhanced the activities of hepatic glycogen synthetase, aspartate and alanine aminotransferases, PC and FDP.

Conversely, dieldrin decreased the activities of hepatic glycogen phosphorylase, glucose-6-phosphatase, hexokinase and glucokinase. Consequently it was concluded that HEOD elevated hepatic glycogen by stimulating gluconeogenic and glycogenic activity in the liver.

The results of Hickenbottom & Yau (1974) contradict those of Bhatia et al. (1973). Hickenbottom & Yau (1974) studied the effects of pp-DDT (50 mg/kg i.p.) on hepatic

glycogen, glycogen synthetase, glycogen phosphorylase and G6P in male Holtzman rats, 18 hours after injection of the insecticide. They found glycogen content and glycogen synthetase activity were decreased by DDT. The pesticide increased glycogen phosphorylase and G6P activities. These effects of DDT were also elicited in adrenalectomized rats. The authors concluded therefore that the effects of pp-DDT were not mediated through the action of adrenal corticoids.

This latter conclusion also refutes that of Bhatia et al. (1972), who explained the effects of HEOD exposure in their Wistar rats on the basis of an observed increase in plasma corticosterone concentration. The source of conflict in the reports cited above may result from many factors. For example, different insecticides and different strains of rats were used in the studies. Kacew et al. (1972a) found the minimal effective per os dose of DDT required to induce the carbohydrate response to be 100 mg/kg. Therefore, it is possible that the 50 mg/kg i.p. dose administered by Hickenbottom & Yau, may have been insufficient to evoke a carbohydrate response at 18 hours. Finally, although both groups examined late-onset effects of the pesticides, Bhatia's determinations were made six hours later than were those of Hickenbottom & Yau.

The results of Bhatia et al. (1973) in regard to glycogen content also differ markedly from those of Kacew & Singhal (1973b). Kacew & Singhal, found hepatic glycogen was decreased in rats given cyclodiene insecticides. However, it should be noted that Bhatia's animals were first treated with HEOD and

then fasted for 24 hours prior to determination of hepatic glycogen content. Kacew & Singhal's rats, on the other hand, were fasted for 16 hours prior to pesticide administration and hepatic glycogen was then assessed after another hour had passed. These procedural differences may account for the disparate results of the two studies.

In 1973, Kacew & Singhal (1973c) first reported the ability of organochlorine pesticides to stimulate, *in vitro*, cAMP synthesis in rat renal cortical tissue. These authors incubated renal cortex tissue in the presence of pp'DDT, chlordane, endrin, dieldrin or heptachlor and measured subsequent synthesis of [3 H]-cAMP from radioactive adenosine. They found that all of the above insecticides caused a significant stimulation in [3 H]-cAMP formation.

Additionally, in pp'DDT-treated incubates, caffeine (a phosphodiesterase inhibitor) potentiated the effect of DDT. Conversely, imidazole (which stimulates phosphodiesterase) reduced the effect. DDT also enhanced cAMP synthesis when either propranolol or hydrazine (which depress glucose synthesis) was present in the incubates. However, prostaglandin E1 or F2 α (which inhibit phosphoenolpyruvate carboxylase) did not alter the stimulatory effect of pp'DDT. Thus it was suggested that the *in vivo* effects of organochlorine pesticides on carbohydrate metabolism may result from pesticide-enhanced cAMP synthesis.

Kacew & Singhal published four papers investigating the effects of organochlorine pesticides on cAMP synthesis and

the insecticide-induced carbohydrate response of the adult Wistar rat. Rather than review these papers individually, the pertinent results are presented below.

Thus,

a) Regarding in vivo pesticide administration:

- i) Renal and hepatic adenyl cyclase (basal and fluoride-stimulated forms) and cAMP concentrations were increased after per os:
 - pp'DDT - (600 mg/kg : Kacew & Singhal, 1973b, 1974b)
 - pp'DDT - (10 mg/100g : Kacew & Singhal, 1974a)
 - chlordane - (200 mg/kg : Kacew & Singhal, 1973b)
 - heptachlor - (200 mg/kg : Kacew & Singhal, 1973b)
 - endrin - (50 mg/kg : Kacew & Singhal, 1973b)
- ii) Renal phosphodiesterase activity was decreased after pp'DDT (600 mg/kg) : (Kacew & Singhal, 1973b, 1974a).
- iii) Renal and hepatic PEPCK, PC, G6P and FDP activities were increased in rats treated with:
 - pp'DDT - (10 mg/100g : Kacew & Singhal, 1974a)
 - cAMP - (10 mg/100g : Kacew & Singhal, 1974a)
 - or pp'DDT (10 mg/100g) and a phosphodiesterase inhibitor theophylline (10 mg/100g : Kacew & Singhal, 1974a)
- iv) [³H]-cAMP synthesis was stimulated in renal and hepatic tissue taken from rats given:
 - pp'DDT - (10 mg/100g : Kacew & Singhal, 1974a)
 - caffeine - (5 mg/100g : Kacew & Singhal, 1974a)
 - pp'DDT (10 mg/100g) and either:
 - caffeine (5 mg/100g),
 - or prostaglandin E1 (10⁴g/100g : Kacew & Singhal, 1974a)
- v) Synthesis of [³H]-cAMP was inhibited in renal cortical tissue obtained from rats treated with pp'DDT (10 mg/100g) and either: imidazole (40 mg/100g), propranolol (0.3 mg/100g) or hydrazine (26 mg/100g) when compared to [³H]-cAMP synthesis in renal tissue from rats given only pp'DDT (10 mg/100g : Kacew & Singhal, 1974a).

- b) In regard to in vitro pesticide administration, the following results were found when renal cortical or hepatic tissue obtained from non-pesticide treated rats was subsequently exposed to organochlorine pesticides:

- i) The presence of pp'DDT (10^{-6} or 10^{-5} M) increased adenylyl cyclase activity in renal (Kacew & Singhal, 1974a, 1974b) and hepatic homogenates (Kacew & Singhal, 1974a).
- ii) The presence of either pp'DDT (10^{-6} M) or caffeine (2.5 mM) in renal cortex incubates increased the rate of [3 H]-cAMP synthesis (Kacew & Singhal, 1974b).
- iii) Renal cortex incubates containing imidazole (25 mM) exhibited a decreased rate of [3 H]-cAMP synthesis (Kacew & Singhal, 1974b).
- iv) Synthesis of [3 H]-cAMP was decreased in incubates containing pp'DDT (10^{-6} M) and either imidazole (10 mM), propranolol (10^{-4} M) or hydrazine (10^{-5} M) (Kacew & Singhal, 1974b).
- v) Caffeine (0.5 mM) in conjunction with a usually non-stimulatory concentration of pp'DDT (10^{-9} or 10^{-8} M) increased the rate of [3 H]-cAMP synthesis in renal incubates (Kacew & Singhal, 1974b).
- vi) Prostaglandin E1 or F2 α (10^{-4} M) did not alter the rate of [3 H]-cAMP in incubates containing pp'DDT (10^{-6} M), when compared to the rate of synthesis caused by pp'DDT (10^{-6} M) alone (Kacew & Singhal, 1974b).
- vii) The presence of pp'DDT (10^{-6} M) increased the rate of [3 H]-cAMP synthesis in hepatic tissue incubates (Kacew & Singhal, 1974b).
- viii) The rate of [3 H]-cAMP synthesis was increased in renal incubates containing 10^{-6} M: op'DDT, α -chlordane, γ -chlordane, heptachlor, heptachlor epoxide, endrin and dieldrin (Kacew & Singhal, 1974b).

Based on these results, Kacew & Singhal (1974a, 1974b)

developed a model in which the effects of organochlorine pesticides on carbohydrate metabolism were mediated through elevated renal and hepatic cAMP concentrations. Thus, according to their model, DDT (for example) binds to some plasma membrane receptor on renal and hepatic cells. This in turn stimulates adenyl cyclase activity which increases intracellular cAMP concentration. The cAMP so produced acts as a second messenger to cause increased activities of: PEPCK, PC, G6P, FDP and glycogen phosphorylase. As an ultimate consequence of this cAMP-mediated metabolic activation, gluconeogenesis and glycogenolysis result. This model therefore explains the hyperglycemia, hyperuremia and decreased hepatic glycogen content typically found after treatment with organochlorine insecticides in the fasted adult rat.

Kohli et al. (1975b) elected to review the "biochemical effects" of DDT and dieldrin. Only those effects not already described and pertinent to altered carbohydrate metabolism will be reiterated here. Thus, according to Kohli et al. (1975b) administration of DDT has:

- a) Increased blood aldolase concentration
- b) Decreased the activity of glucose-6-phosphate dehydrogenase
- c) Decreased the concentration of hepatic NAD, NADP, NADH and NADPH
- d) Increased the activity of hepatic NAD hydrolase.

Similarly, treatment with HEOD has:

- a) Inhibited the activities of hepatic mitochondrial isocitrate dehydrogenase and succinic acid dehydrogenase

- b) Decreased the rate of hepatic mitochondrial respiration by inhibiting the electron transport chain at the level of cytochrome b. (This effect was not HEOD-dose dependant)
- c) Increased the activity of the enzymes of the hexose-monophosphate shunt pathway.

Kohli et al. (1975b) stated that due to the lack of consistency in the reviewed studies in regard to species, sex, age, strain and nutritional status of the animals employed and the amounts, types and dose regimes (i.e. chronic or acute) utilized for the two insecticides, they were unable to "ascribe the mode of action or the current of events which follow the administration of the insecticide (s)". In regards to specific effects on carbohydrate metabolism, these authors concluded only that, "Insecticides in general, enhance gluconeogenesis in liver and kidney. (sic) Dieldrin also increases the activity of the hexose-monophosphate shunt pathway."

Story et al. (1976) studied the effects of DDT on gluconeogenesis in adult rat liver tissue. These authors, in contrast to Kacew's group, utilized perfused rat livers to perform their study. Briefly detailed, these workers fed op DDT (1000 ppm in rat-chow) for 12 days to rats. The animals were then fasted for 48 hours prior to sacrifice and in situ perfusion of the livers. No explanation was offered by these investigators as to why their animals were fasted for such an extensive time before sacrifice. Story et al. (1976) found that gluconeogenesis from 10 mM lactate was decreased in the in situ perfused livers of DDT-fed rats.

In other experiments utilizing isolated hepatocytes from

their DDT-fed rats, gluconeogenesis from 9 mM lactate:1 mM pyruvate, was also reduced (by 25%). Conversely, hepatocellular gluconeogenesis from 10 mM glycerol or 10 mM alanine was unchanged by DDT. Additionally, the levels of hepatocellular gluconeogenesis from 9 mM lactate:1 mM pyruvate induced by either 50 μ M glucagon or 2 mM butyrate, were equivalent in both DDT-ration fed and control-ration fed rats. The authors concluded that chronic DDT feeding decreased gluconeogenesis in hepatocytes obtained from 48 hour starved rats.

Only two papers of interest in this review, were published in 1977. The first by Yau and Mennear (1977) examined the inhibitory effect of DDT on insulin secretion in mice. These authors treated adult Swiss albino mice with a single oral dose of DDT (50 mg/kg). Blood samples from these animals were collected at 0, 1, 3, 5, 7 and 18 hours after DDT ingestion, for assay of serum glucose and immuno-reactive insulin (IRI). No hyperglycemia was found in the DDT-exposed mice at any time tested; in fact, a slight hypoglycemia was observed at 5 and 7 hours. Serum IRI concentration was only determined at 18 hours and this also was unaffected by DDT.

Yau and Mennear (1977) also performed glucose tolerance tests on DDT-treated mice. The animals were pretreated with DDT (50 mg/kg p.o.) and 18 hours later received a loading dose of glucose (2 g/kg i.p.). DDT significantly reduced the glucose tolerance of the mice. Pancreatic islets were also isolated from mice treated 18 hours previously with DDT. These isolated islets were incubated in the presence of either

d-glucose (3 mg/ml of incubate) or tolbutamide (0.8 mg/ml of incubate) and the levels and rates of released IRI were measured. Both d-glucose and tolbutamide increased the release of pancreatic insulin.

In pancreatic islet incubates from DDT-treated animals, the amount and rate of IRI released were significantly reduced in the presence of d-glucose (i.e. a 66% reduction in the amount of IRI found at 90 minutes) or tolbutamide (i.e. a 55% reduction at 90 minutes). Yau and Mennear (1977) concluded that DDT inhibited pancreatic β -cell secretory activity in the face of stimulatory challenge in their mice. These authors considered such inhibition to be consistent with known DDT-induced stimulation of hepatic gluconeogenic activity, since the activities of hepatic gluconeogenic enzymes and glycogen phosphorylase were increased in the absence of insulin. The lack of any hyperglycemic response or any inhibition of IRI release in the absence of stimulatory challenge observed in vivo in the DDT-treated mice, probably resulted as a result of the low dose of DDT employed. Thus, the authors believe that 50 mg/kg p.o. DDT represents a near minimum threshold dose for 18 hour DDT-induced inhibition of pancreatic activity.

The second paper of 1977, that of Aravindakshan et al. (1977) examined the effect that chronic pp-DDT feeding had on serum and hepatic transaminase activities in the rat. These authors fed pp-DDT to albino rats in different amounts over a six month period.¹ At the end of six months, the rats were

1. The increasing dietary concentrations of pp-DDT fed over six consequent months were: 50, 100, 150, 200 and 300 ppm/month.

killed and serum and hepatic glutamic pyruvic and glutamic oxaloacetic transaminase activities were measured. The pesticide increased body and liver weights and the concentrations of pp'DDT and DDE present in "body fat". Hepatic and serum transaminase activities were unchanged.

Story and Freedland (1978a) reported the results of their continued studies into the effects of DDT on carbohydrate metabolism in isolated rat hepatocytes. They fed 1000 ppm pp'DDT to rats for two weeks, and then fasted these rats for 48 hours prior to isolating hepatocytes for study. As in their previous report (Story et al., 1976) it was found that the rate of hepatocellular gluconeogenesis from 10 mM lactate:pyruvate (9:1) and the percent level of stimulation of gluconeogenesis from lactate:pyruvate induced by the presence of glucagon (10^{-9} to 10^{-6} M) in the hepatocellular incubates, were reduced by DDT. Additionally, when lactate:pyruvate and 10^{-7} to 10^{-5} M cAMP were present together in the incubates the rate of gluconeogenesis and the percent stimulation of gluconeogenesis induced by cAMP were both decreased in cells taken from rats fed DDT.

However, when the concentration of cAMP in the system was raised to 10^{-4} M, "DDT" hepatocytes exhibited gluconeogenic stimulation which was equivalent to that seen for "control" hepatocytes. The authors concluded that the primary effect of DDT might be to alter the gluconeogenic response of hepatocytes to the level of intracellular cAMP and not to alter the mechanism by which glucagon increases the level of cAMP.

Story & Freedland (1978b) examined the effects, again in isolated hepatocytes, that op-DDT feeding had on gluconeogenesis and the activities of some gluconeogenic enzymes. Adult male Sprague-Dawley rats were fed a diet containing 1000 ppm op-DDT for a period of two weeks. This regime produced an average daily intake of 150 mg/kg op-DDT in the animals. At the end of the feeding period the rats were starved for 48 hours to ensure that maximal mobilization of DDT from adipose tissue would occur. The animals were then killed to obtain hepatocytes. The results found by these authors are enumerated in some detail below.

- a) DDT feeding did not alter weight gain or food consumption in the rats.
- b) Hepatocellular gluconeogenesis from 10 mM lactate or 9 mM lactate:1 mM pyruvate was decreased in DDT-fed rats.
- c) Hepatocellular gluconeogenesis from 10 mM, alanine, fructose or glycerol was unaltered by prior DDT exposure.
- d) The presence of 2 mM butyrate in incubates of either "DDT" or "control" (non-DDT fed) hepatocytes caused an equivalent percent stimulation in the rate of gluconeogenesis from 9 mM lactate:1 mM pyruvate. However, the absolute rate of gluconeogenesis produced was significantly lower in the DDT than in the control incubates.
- e) When control cells were incubated with 9 mM lactate:1 mM pyruvate and 10^{-7} M DDT, no effect was produced on gluconeogenesis.
- f) The authors measured [14 C]-bicarbonate fixation in intact mitochondria and the activities of mitochondrial PC and cytosolic PEPCK in liver tissue of DDT fed rats. Mitochondrial CO_2 fixation and PC activity were unchanged as a result of DDT. However, hepatic PEPCK activity was significantly decreased by the pesticide.
- g) The authors also added either 2 mM oleate or 2 mM oleate and 9 mM lactate:1 mM pyruvate to control and DDT hepatic

incubates, and measured the rate of ketogenesis and the β -hydroxybutyrate:acetone ratios produced. Under either condition, equivalent ketogenic rates were found for both DDT and control cells. However, in both cases DDT decreased the β -hydroxybutyrate:acetone ratio.

Story & Freedland (1978b) concluded on the basis of these results that prior DDT exposure in the 48 hour starved rat had no effect on gluconeogenic pathways leading from alanine, fructose or glycerol. The observed DDT-induced inhibition of gluconeogenesis from lactate:pyruvate did not result from an inability to oxidize fatty acids (e.g. butyrate) and thus produce acetyl-Co-A, nor from any impairment of PC function. The addition of op-DDT (10^{-7} M) to hepatocellular incubates obtained from non-DDT fed rats did not alter normal gluconeogenesis. The pesticide did decrease hepatic PEPCK activity. However the attenuation was not of sufficient magnitude to explain the decreased rate of gluconeogenesis from lactate found in hepatocytes obtained from DDT-exposed rats. DDT did not alter ketogenesis from oleate, a fatty acid which requires carnitine acyl transferase transport into the mitochondria. Therefore, fatty acid transport into hepatic mitochondria was not altered by the pesticide. Finally, a more oxidized hepatic mitochondria redox state was produced by DDT exposure, since the β -hydroxybutyrate:acetone ratio was reduced. However, this effect was not thought to be responsible for the decrease in the rate of gluconeogenesis from lactate caused by DDT.

The final summary conclusion reached by these authors was; "DDT feeding inhibits gluconeogenesis from lactate between pyru-

vate and phosphoenolpyruvate".

Story & Freedland (1979) studied the effects of DDT feeding, on gluconeogenesis from lactate in isolated hepatocytes incubated in the presence of glucagon or dibutyryl cAMP. Experimental conditions used in this study were identical to those described in the 1978 study (Story & Freedland, 1978b).

The authors were able to show that maximal rates of hepatocellular gluconeogenesis from 9 mM lactate:1 mM pyruvate resulted in control (non-DDT fed) hepatocellular incubates in which 10^{-6} M glucagon or 10^{-5} M dibutyryl cAMP were present. Conversely, in "DDT" hepatocellular incubates, containing either glucagon or cAMP, both the absolute rate of gluconeogenesis and the percent stimulation of gluconeogenesis from 9 mM lactate:1 mM pyruvate were decreased.

If the concentration of glucagon was raised to 5×10^{-5} M, an equivalent percent increase in stimulation of gluconeogenesis occurred in both control and DDT hepatocytes. However, the absolute rate of gluconeogenesis was less in the DDT cells than in the control cells.

The authors found that this inhibitory effect of DDT on gluconeogenesis was specific for lactate. Thus hepatocellular gluconeogenesis from 10 mM pyruvate was equivalent in both DDT and control cells. Also measured were the activities of liver lactate dehydrogenase and liver mitochondrial and cytosolic aspartate aminotransferases. DDT feeding had no effect on the activities of these enzymes.

Story & Freedland (1979) concluded that DDT feeding

decreased the sensitivity of rat hepatocytes to glucagon and dibutyryl cAMP. The decreased sensitivity to glucagon could be overcome provided glucagon concentration was sufficiently high (i.e. 5×10^{-5} M). Therefore, it was suggested that the inhibition of lactate:pyruvate gluconeogenesis seen in DDT cells resulted from a DDT-induced alteration of the normal response of these cells to cAMP and not from some DDT-induced alteration in the mechanism by which glucagon increases intracellular cAMP concentration. Finally, despite a lack of DDT altered aspartate aminotransferase activity, the authors believed that the step in gluconeogenesis from lactate which was by and large inhibited by DDT was in fact that of aspartate transport out of the mitochondrion.

Story et al. (1982) reported the effects of DDT feeding on nitrogen metabolism in the 48 hour starved rat. Again, Sprague-Dawley rats were fed 1000 ppm op DDT for two weeks prior to experimentation. Briefly summarized, the authors found that DDT feeding decreased hepatocellular urea synthesis from 5 mM ammonium chloride and 10 mM ornithine. However, this effect only occurred in animals which had been starved for 48 hours. Urea synthesis from 10mM alanine, serine or threonine was not altered by DDT. The pesticide did not increase the mortality of 48 hour starved rats given ammonium chloride (5 mmol/kg i.p), nor did it alter in vivo plasma urea concentrations in these animals.

A decreased rate of hepatocellular urea synthesis from ammonium chloride:ornithine was also found in cells obtained

from rats fed a diet of 90% casein supplemented with 1000 ppm DDT for 11 days. Thus the authors concluded that DDT feeding decreased the capacity for urea synthesis in hepatocytes from both 48 hour starved and high-protein diet fed rats. However, this reduction in urea synthetic capacity did not significantly alter either in vivo ammonia detoxification or toxicity.

By this point the reader has probably formed the opinion that much of the work of Story's group might contradict that of Kacew's group. This is perhaps an opportune time to re-emphasize that Story's experiments refer primarily to in vitro studies of carbohydrate metabolism in hepatocytes of DDT-fed, 48 hour starved Sprague-Dawley rats, whereas Kacew's work by and large, relates to in vivo studies of the effects of a single dose of an organochlorine pesticide on carbohydrate metabolism in 16 hour post-absorptive Wistar rats. Consequently, many of the disparate results found by these two major groups of investigators may reflect technical as opposed to true pharmacological and/or physiological differences in the effects of these agents on rat carbohydrate metabolism.

No further study of altered carbohydrate metabolism following organochlorine exposure in the rat ~~per se~~ has been published since 1979. However, Bandyopadhyay et al. (1982a) reported increased G6P activity after dieldrin treatment in the rat. This result was found as part of these authors' study of the effects of l-ascorbic acid supplementation on chronic HEOD toxicity.

Chronic exposure to HEOD (5 mg/kg/day for 15 days) in

Juvenile male Wistar rats also caused renal and hepatic necrosis, increased liver ascorbic acid concentration and elevated the activities of hepatic G6P and l-gulonolactone oxidase and brain inorganic pyrophosphatase. The regime also decreased the activities of hepatic Mg^{+2} -adenosine triphosphatase and brain acetylcholinesterase.

When l-ascorbic acid supplements (20 mg/100g/day p.o. for 15 days) were fed concurrently with HEOD, renal and hepatic pathology was ameliorated to some extent. These supplements also increased hepatic ascorbic acid content and caused a further augmentation in HEOD-elevated hepatic G6P and l-gulonolactone oxidase activities. Conversely, the supplements restored to control levels the activities of hepatic Mg^{+2} -adenosine triphosphatase and brain inorganic pyrophosphatase in HEOD-exposed rats. Finally, brain acetylcholinesterase activity was further decreased in rats given both l-ascorbic acid and HEOD.

The authors concluded that l-ascorbic acid supplementation was an effective treatment which could prevent some of the pathology and altered enzymatic function which normally develops in the rat chronically exposed to dieldrin.

Bandyopadhyay et al. (1982b) also studied the effects of chronic HEOD with and without l-ascorbic acid supplementation on rat hepatic plasma membrane enzymes. Young male Wistar rats were exposed to HEOD alone (5 mg/kg/day p.o. for 15 days) or HEOD and l-ascorbic acid (20 mg/100 g/day p.o. for 15 days).

Dieldrin alone decreased the activity of hepatic Mg^{+2} -

adenosine triphosphatase and increased the activities of hepatic 5'-mononucleotidase and NADH dehydrogenase. 1-Ascorbic acid given in conjunction with HEOD restored the activity of Mg^{+2} -adenosine triphosphatase to that seen in control (non-HEOD treated) rats. However, the vitamin was unable to prevent HEOD-elevated hepatic 5'-mononucleotidase or NADH dehydrogenase activities.

The effects of DDT on the activity of another rat hepatic plasma membrane enzyme, adenylyl cyclase, have also been studied recently. Poschl et al. (1982) treated overnight fasted adult male Wistar rats with DDT (isomer not stated) either acutely (500 mg/kg p.o.) or chronically (25 mg/kg/day p.o.) for six days. Acutely dosed animals were killed to obtain liver tissue two hours after the administration of the pesticide. Rats treated chronically were killed one day after receiving their final dose of DDT.

Neither acute nor chronic DDT exposure altered the basal activity of hepatic adenylyl cyclase, nor was the percent stimulation in cyclase activity induced by glucagon changed as a consequence of DDT exposure. Also, neither DDT treatment was able to alter the activity of hepatic Na^{+}/K^{+} adenosine triphosphatase. On the other hand, the presence of either 20 or 100 μM DDT in hepatocellular homogenates obtained from non-DDT exposed rats, reduced glucagon-induced stimulation of adenylyl cyclase activity by 20 and 40% respectively. However, even in these preparations, the basal level of adenylyl cyclase activity was unaltered by the presence of DDT.

The authors also determined the concentration of [^{14}C]-DDT in hepatic homogenates obtained from overnight fasted rats two hours after per os administration of 500 mg/kg [^{14}C]-DDT. Typically, such a rat liver homogenate contained a total of 590 μg [^{14}C]-DDT or a concentration of 0.21 μg [^{14}C]-DDT/mg hepatic protein. This [^{14}C]-DDT was not uniformly distributed throughout the hepatocyte, since 10 fold more [^{14}C]-DDT was associated with cell membranes than with any other component.

Finally, these authors stated that the degree of adenylyl cyclase stimulation (i.e. the ratio of fluoride-stimulated activity to basal activity) calculable for the results of Kacew & Singhal's studies (1973b, 1974b), was not significantly altered as a result of treating rats with pp-DDT (600 mg/kg p.o.). It should be noted that Poschl et al. (1982) presented no statistics to support this latter conclusion.

The final conclusion reached by Poschl et al. (1982) was that their results "do not support the assumption that DDT stimulates adenylyl cyclase activity in rat liver".

In vitro Na^+/K^+ adenosine triphosphatase activity was also decreased in rat small intestine homogenates containing 10^{-5} M DDT or 10^{-4} M DDE (dichloro-diphenyl-dichlorethylene), (Iturri & Wolff, 1982). These authors also found the above concentrations of DDT and DDE could inhibit in vitro active transport of d-glucose and l-tyrosine in rat small intestine.

B. The neonate.

Very little is known about the effects of the organochlorine pesticides on carbohydrate metabolism in the neonatal

mammal. Only one paper to date has addressed this aspect.

Thus, Costella & Virgo (1988) found that in mice, in utero exposure to dieldrin induced a congenital inviability in caesarian delivered pups. In utero pesticide exposure was achieved by exposing dams to HEOD (2 mg/kg/day p.o.) from day 6 to day 18 of their pregnancy.

Inviabile pups were hypoglycemic at the time of their delivery when compared to viable control (non-HEOD exposed) pups. These pups were still hypoglycemic 19 hours later and were aglycemic by 24 hours. Hepatic glycogen content was equivalent in both viable and inviable pups at the time of delivery. However, glycogen concentration decreased more rapidly post-partum in the inviable than in the viable pups (i.e. a 77% difference at 24 hours). The glycogen content of both cardiac and gastrocnemius muscle was also reduced in HEOD-exposed pups at 24 hours. In utero HEOD exposure did not alter the activities of hepatic lactic dehydrogenase or FDP at 0, 19 or 24 hours post-partum, nor were the blood concentrations of urea, lactate or pyruvate altered at these times in the inviable pups.

Thus, Costella & Virgo (1988) showed that HEOD exposure potentiated normal post-partum hypoglycemia and increases the rate of hepatic glycogenolysis in fasted neonates. Also, the authors indicated that in the inviable pup, once blood glucose had fallen below a critical level, the utilization rate of glycogen in cardiac and skeletal muscle was increased. The lack of any significant effect of HEOD to alter at any time

the gluconeogenic parameters studied was considered indicative of the existence of normal gluconeogenic capacity in the inviable pups.

These results regarding carbohydrate metabolism after organochlorine pesticide exposure in the neonatal mouse are largely opposite to those reported by Kacew or Bhatia for the adult rat. For example, both Kacew & Singhal (1973b) and Bhatia et al. (1973) found hepatic FDP activity to be increased after exposure to cyclodienes. In contrast to the neonatal mouse, blood lactate and pyruvate concentrations were increased in the adult rat following HEOD (Bhatia et al., 1972). Similarly, hyperuremia was found after cyclodiene exposure in the adult rat (Kacew et al., 1973; Kacew & Singhal, 1973b, 1973c, 1973d).

In regard to hepatic glycogen content, Kacew et al. (1973b) and Kacew & Singhal (1973d) found this parameter was decreased following cyclodiene treatment in the adult. Conversely, Bhatia et al. (1973) reported elevated hepatic glycogen concentration in their dieldrin treated adults. Thus, Costella & Virgo's conclusion of enhanced glycogenolysis in the HEOD exposed new-born mouse agrees with that of Kacew's group but refutes that of Bhatia's.

Costella & Virgo's results show better agreement to those reported by Story's group who studied the effects of DDT-feeding on carbohydrate metabolism, in hepatocytes obtained from adult rats. For example, Story et al. (1976) found gluconeogenesis from lactate was decreased in DDT hepatocytes. If

a similar situation exists in vivo in the neonatal mouse, it might explain why the neonate lacks a HEOD-induced hyperglycemic response. Story et al. (1976) and Story & Freedland (1978b) also reported that hepatocellular gluconeogenesis from glycerol was unaltered by DDT. This result suggests that FDP activity may not be affected by DDT in the adult rat. Story & Freedland (1979) found unaltered hepatic lactate dehydrogenase activity in hepatocytes from DDT-fed rats. This result is similar to Costella & Virgo's report of unaltered blood lactate dehydrogenase in the HEOD-exposed neonatal mouse. Finally, Story et al. (1982) detected no change in serum urea concentration in vivo in their DDT-fed adult rats.

Thus, Costella & Virgo's (1980) results can both be supported and refuted on the basis of the adult literature. Consequently, at this point it is perhaps prudent to merely conclude that the effects on carbohydrate metabolism of organochlorine pesticide exposure differ in the neonate and the adult.

C. Adult animals (other than the rat).

The effects of organochlorine pesticides on carbohydrate metabolism have not been studied solely in the rat. During the last five years, several investigators have studied the effects of pp-DDT and dieldrin in the rhesus monkey (Macaca mulatta). These studies will not be reviewed in any detail here. However, the following is a summary of pertinent results found regarding carbohydrate and lipid metabolism.

Thus a single dose of pp-DDT (150 mg/kg p.o.) 48 hours after administration to the rhesus monkey caused:

- a) increased serum activities of glutamic oxaloacetic transaminase, glutamic pyruvate transaminase, alkaline phosphatase, amylase and lactic dehydrogenase :
(Agarwal et al., 1978).
- b) no alteration in blood glucose, urea and lactate concentration or the activity of serum aldolase :
(Dudeja et al., 1980).
- c) a two-fold increase in hepatic glycogen content :
(Dudeja et al., 1980).
- d) increased activities of hepatic glycogen synthetase and G6P : (Dudeja et al., 1980);
and glutamic oxalase transaminase, succinate dehydrogenase and lactic dehydrogenase of liver, kidney and spleen :
(Agarwal et al., 1978).
- e) increased activities of hepatic and renal Mg^{+2} -adenosine-triphosphatase and amylase : (Agarwal et al., 1978).
- f) decreased activities of hepatic glycogen phosphorylase and adenylyl cyclase (basal, and fluoride and epinephrine stimulated forms) : (Dudeja et al., 1980);
and hepatic alkaline phosphatase: (Agarwal et al., 1978).
- g) no alteration in the activities of hepatic acid phosphatase: (Agarwal et al., 1978)
or hepatic FDP : (Dudeja et al., 1980).
- h) no alteration in the hepatic concentration of protein, RNA, DNA or lactic acid : (Dudeja et al., 1980).

Similarly, a single dose of dieldrin (20 mg/kg p.o.), 24 hours after administration to the rhesus monkey produced the following effects on lipid metabolism and the intestinal transport of glucose:

- a) increased the triglyceride content in liver, kidney and adipose tissue : (Agarwal et al., 1981).
- b) increased the incorporation of [^{14}C]-glucose into hepatic triglyceride and produced histological evidence of hepatic "fatty" degeneration : (Agarwal et al., 1981).
- c) increased the activity of lipoprotein lipase in liver, heart and adipose tissue and post-heparin lipase in plasma: (Agarwal et al., 1981).
- d) did not alter plasma lipid concentration or alter the rate of fat clearance in the intravenous fat tolerance test :
(Agarwal et al., 1981).

- e) increased the rate of uptake of d-glucose and decreased the rate of uptake of leucine in vitro in small intestine : (Mahmood et al., 1981).
- f) increased the activities of intestinal sucrase, lactase, maltase and alkaline phosphatase and decreased the activity of leucine amino peptidase in small intestine : (Mahmood et al., 1981).

A final example of the apparent ability of organochlorine insecticides to alter carbohydrate metabolism in many different species is forthcoming from the study of Srivastava & Singh (1981). These authors found that carbohydrate metabolism was impaired in the Indian catfish (Heteropneustes fossilis) after exposure to aldrin.

The fish were exposed to a 0.1A ppm concentration of aldrin in fresh water for a period of 96 hours. The aldrin concentration used was equivalent to 80% of the experimentally determined LC50 (i.e. lethal concentration for 50% of an exposed population). Liver and skeletal muscle glycogen contents and blood glucose, lactate and pyruvate concentrations were measured in the exposed fish at 3, 6, 12, 48 and 96 hours.

Aldrin decreased glycogen content in liver at three hours and in muscle at 3, 48 and 96 hours. Hyperglycemia was found at all times examined throughout the experiment. Finally, blood lactate concentration was increased at 3 and 12 hours, while elevated blood pyruvate levels occurred at 3, 6 and 96 hours. The authors speculated that the observed hyperglycemia resulted from aldrin-enhanced hepatic glycogenolysis, but concluded only that the pesticide had impaired normal carbohydrate metabolism.

MATERIALS AND METHODS

A. General materials and methods.

Ai. Chemicals.

Appendix 1 presents a list of the chemicals used, and their source. All chemicals were analytical grade (AR) or better.

Aii. Technical notes and procedures common to all experiments.

Unless otherwise stated, the following apply to all experiments described herein.

1. Dieldrin is the generic name for the insecticide, 1,2,3,4,10,10,-hexachloro-6,7,-epoxy-1,4,4a,5,6,7,8,8a-octa-hydro-endo-exo-1,4:5,8-dimethanonaphthalene. Consequently, the abbreviation HEOD is used to indicate dieldrin.
2. "Adult" male rat refers to a 60 day old rat.
3. The purity of the HEOD used in experiments was 98.5%.
4. HEOD was dissolved in corn oil and administered (per os).
5. Five and ten day old rat pups were fasted for three hours prior to use. This allowed at least partial digestion of any milk present in the animal's stomach.
6. Adult rats were subjected to the following fasting regime prior to use: fasted six hours, fed six hours, and fasted again for 12 hours. This regime ensured: a) that the gut would be essentially empty, permitting consistent absorption of the HEOD, and b) that blood glucose level and hepatic glycogen content would be similar in all rats.
7. Animals were weighed at the end of the fasting period to allow calculation of an appropriate HEOD dose to be made.
8. Food was withheld but tap water was provided ad libitum to adult rats during the experiments.
9. Experiments were performed with animals from at least two, and usually more, litters.
10. Tail blood samples were used solely for blood glucose analyses.

11. Liver samples were analysed for glycogen and PEPCK only.
12. All spectrophotometry was done on a Perkin-Elmer, Coleman 575 double-beam spectrophotometer (Coleman Instruments Div., Oak Brooks, Ill.).

Aiii. Animals.

Woodlyn Wistar I rats, derived from the Carworth Wistar, were purchased from Woodlyn Laboratories Ltd. (Guelph, Ont.), and used to establish a breeding colony in our animal quarters. The colony was maintained under a constant photoperiod of 12 hours of light (lights on at 0800 hours) in quarters at 21° C. Purina Lab Chow and tap water were provided ad lib. Breeding animals were caged in suspended, stainless steel mesh cages - three females and one male per cage. Pregnant females were transferred to 30x33x15 cm plastic solid-bottom cages, on the 16 - 18th day of pregnancy, and supplied with hardwood bedding (Betta Chip - Northeastern Products, Warrensburg, N.Y.). These females were allowed to deliver and their litters were reduced to 10 pups within 12 hours of birth. The females were then allowed to raise the pups until they either: a) were used experimentally (5 - 10 days of age) or b) were weaned (22 - 24 days of age).

All experiments with pups or adult rats over 60 days of age were conducted with "colony - produced" animals. Most experiments with 60 day old adults were also conducted with colony-produced animals, but some employed animals purchased from Woodlyn Laboratories Ltd..

B. Acute toxicity of HEOD in: 5, 10 and 60 day old rats.

The dose of HEOD required to kill 50% of a test popula-

tion (LD50) was determined in five and ten day old pups (mixed sexes) and in 60 day old adult males.

Bip. HEOD LD50 determination in five and ten day old pups.

Five or ten day old pups from five to seven different litters were separated from their dams and fasted in a Blue M Electric Co. Model 200A incubator (Blue Island, Ill.) at $28 \pm 1^{\circ}$ C. for three hours. Prior experimentation had shown that the temperature beneath a dam and its pups was $30 \pm 2^{\circ}$ C. After fasting, the pups were randomly assigned to subgroups, and treated with HEOD. The following doses were given, per os:

to five day old pups: 5, 15, 25, 35, 45, 55, 65, 75 and 85 mg/kg;

to ten day old pups: 10, 15, 20, 25, 30, 35, 50 and 75 mg/kg.

The total number of pups at each dose ranged from 5 to 35, with approximately 25 being used at each dose in the linear response range (ie. 15 - 45 mg/kg).

The pups were then caged individually in the incubator for the next 24 hours. In addition to the HEOD-treated pups, oil-treated animals served as a control group. The number of dead pups were counted every six hours and the cumulative mortality was calculated at the end of 24 hours. No mortality occurred within the control pups.

In order to process the number of pups required for statistically valid estimates of the LD50, the above procedure was repeated several times over a period of several weeks.

Bii. HEOD LD50 determination in 60 day old males.

The procedure used to determine the acute toxicity of HEOD in the 60 day old male rat was similar to that described above. The actual procedure was as follows.

At 59 days of age, littermates were fasted and randomly assigned to treatment groups. Usually rats from two to three different litters were tested simultaneously. The animals were weighed and treated per os with HEOD at 25, 50, 60, 70, 100, 115 or 125 mg/kg. Three to twelve rats were tested with each dose; approximately eight rats were given each dose in the linear response range (ie. 50 - 100 mg/kg). Oil-treated animals served as controls. After HEOD treatment, the rats were caged individually and the mortality was determined after 24 hours. No mortality occurred in the controls.

The LD50 determination for adult males was repeated five times over a period of five days to ensure an accurate estimate. The resulting data, as in the case for the five and ten day olds, were analysed according to the method of Litchfield and Wilcoxon (1949).

C. Determination of: plasma glucose, plasma free fatty acids and hepatic glycogen, one and three hours after HEOD.

A series of experiments, designed to determine the effects of HEOD on plasma glucose, plasma free fatty acid (FFA) levels and hepatic glycogen content, in 5, 10 and 60 day old rats were performed. Preliminary experiments indicated that HEOD (at a dose of one-half LD50) would produce a peak hyperglycemic response in adult rats approximately two hours after exposure.

Consequently, substrate parameters were assessed, one and three hours post-treatment. The details of sample procurement and analysis follow.

Ci. Five and ten day old pups - sample collection.

The procedure below was applied to both five and ten day old pups. Pups were fasted and then randomly assigned to either a control or treatment group. Treatment pups were given HEOD (at a dose equal to either one-half or one age-specific LD50). The control pups were given appropriate volumes of the corn oil vehicle. Pups of both groups were then individually caged and maintained at 28° C in an incubator for one or three hours. The animals were then removed and processed as follows.

Each pup was decapitated and arteriovenous trunk blood was collected in a clear plastic weight-tray containing 0.1 ml of a 1000 U/ml heparin solution. The heparinized blood was then transferred to a clean dry 13 x 100 mm test-tube and placed in ice for 30 minutes while the liver samples were collected. The cooled blood samples were then centrifuged for five minutes at 3000 rpm in an I.E.C. model K centrifuge, (Needham Hts, Mass.). The resulting plasma samples were transferred to 13 x 100 mm. test tubes, parafilm sealed and stored frozen (-20° C) for blood glucose and FFA analysis. The liver was excised after exsanguination and approximately 100 mg, for glycogen analysis, was weighed on a Roller Smith precision balance, (Biolar Corp., North Grafton, Mass.). The sample was then frozen in liquid nitrogen (-196° C), transferred to a 15 ml conical centrifuge tube, parafilm sealed and stored frozen at -20° C.

Cii Adult males - sample collection.

Methods for obtaining blood (for blood glucose and plasma FFA determination) and liver (for hepatic glycogen content) from adult male rats, were similar to those employed for the pups. Thus, 60 day old males were fasted, divided into control and treatment groups, treated with HEOD (i.e. one-half or one LD50) or oil, and decapitated one or three hours later. The trunk blood was collected and heparin added (0.1 ml of 1000 U.S.P. units/ml aqueous heparin /ml of whole blood). Next, two samples of approximately 100 mg each were taken from the distal end of the anterior lobule of the right lateral lobe of the liver, (Greene, 1955). All samples were then processed in identical fashion to those obtained from the pups.

Dieldrin's effects on blood glucose, FFA and hepatic glycogen were also studied in 120 and 200 day old male rats. The 120 day old animals were given HEOD (either 31.5 or 63 mg/kg) and were killed, one or one and one-half hours respectively post-treatment. The 200 day old rats received HEOD (31.5 mg/kg) and were sacrificed one and one-half hours after treatment. The procedures used to fast, gavage and to collect blood and liver samples were identical to those employed for 60 day old rats.

Ciii Analytical procedures.

a) Plasma glucose.

The quantitation of plasma glucose was performed on 20 μ l samples of thawed plasma, according to the method of Kingsley and Getchell (1960). In that method, glucose is oxidized to

gluconic acid and hydrogen peroxide by glucose oxidase. The peroxide then oxidizes o-dianisidine in the presence of horseradish peroxidase to form an amber coloured product. Finally, 50% (w/v) sulfuric acid is added to give a stable rose coloured product. The absorbance of that product was then measured at 530 nm.

Samples were analysed simultaneously with aqueous reference standards, (25 to 300 mg/dl). Plasma glucose concentrations, expressed as mg glucose/dl plasma were calculated from a standard curve.

b). Plasma FFA.

Plasma FFA was measured in 100 μ l samples of thawed plasma, using a modification of the method of Soloni & Sardina (1973). In this method, plasma FFA is saponified in the presence of copper to form a copper soap, which is then extracted into chloroform. To the chloroform extract is added cuprizon (i.e. oxalic acid bis-cyclohexylidene hydrazide) and ammonia. At basic pH the cuprizon-copper soap complex develops a stable blue colour, the absorbance of which may be measured at 620 nm.

The following modifications were made to Soloni and Sardina's method.

- 1) Acetone-rinsed 16 x 125 mm disposable test tubes were used.
- 2) A total of three mls of chloroform was added to each extraction tube.
- 3) Extraction tubes (containing 100 μ l of thawed plasma, 0.3ml of copper reagent and 3 ml of chloroform) were placed into a rack which had been previously secured to a Lab-line model

3520 orbital shaker (Lab-line Instruments Inc, Melrose Park, Ill.) and shaken at 300 rpm for 30 minutes.

- 4) Following extraction, the organic layer was transferred to a second clean 16x125 mm tube (drying tube).
- 5) To each drying tube was added a "concertina-folded" strip of Whatman #1 filter paper with a surface area of approximately 4.0 sq.cm. The paper absorbed any residual aqueous fraction present in the organic phase.
- 6) After "drying", 2.0 mls of the chloroform extract was transferred to a third clean 16x125 mm tube (assay tube).
- 7) To the assay tube were added 1.8 ml of cuprizon reagent; this was followed by gentle hand shaking.
- 8) Finally, 0.2 ml of the ammonium solution was added to the assay tube and the tube was again gently shaken. Tubes were allowed to stand 15 minutes prior to absorbance measurement to ensure complete colour development.

Samples were analysed simultaneously with reference standards (0.5 to 5.0 mg of bovine serum albumin-bound palmitic acid). Plasma FFA concentrations, expressed as mg of palmitic acid/dl of plasma, were calculated from a standard curve.

c) Hepatic glycogen.

The glycogen content of the liver samples was determined by the method of Seifter et al. (1950). In that method, liver is digested in hot potassium hydroxide and the glycogen is then precipitated with ethanol. The glycogen precipitate is then dissolved in water and an aliquot of the solution is added to 0.2% anthrone in 95% (v/v) sulphuric acid. The acid formed glycogen furfural derivative condenses with anthrone to form a stable blue compound. This absorbance of this compound may then be measured at 620 nm, to give a measure of glycogen present in the sample solution.

Thus, sample aliquots were analysed simultaneously with

reference standards (0.5 to 50.0 mg rabbit glycogen). Glycogen concentrations in the sample aliquots were calculated from a standard curve. From these concentrations, total glycogen content of the sample solutions were calculated. Finally, for each sample, the total glycogen content was divided by the mass of liver used. Thus the glycogen level is expressed as μg glycogen/mg wet liver weight.

D. Determination of gluconeogenic enzyme activities in rat liver.

The effects of HEOD on hepatic fructose-1,6-diphosphatase (FDP), glucose-6-phosphatase (G6P) and phosphoenolpyruvate carboxykinase (PEPCK) activities was studied in five and ten day old pups and in adult rats. These activities were measured one and three hours after HEOD administration (dose: one-half or one age-specific LD50). Plasma glucose levels were also determined at these times. The subsections below give the details of sample procurement and enzyme activity analysis. Blood sampling and plasma glucose analysis were performed as described in section C.

Di. Hepatic enzyme studies - sample collection.

Rats were fasted and then gavaged with either HEOD (dose: one-half or one age-specific LD50) or corn oil. After one or three hours, the animals were decapitated and blood samples were collected. The liver was then excised, blotted dry, rapidly weighed and transferred to a prechilled beaker to which ice-cold 0.25 M sucrose was added (10 volumes for pup liver; 5 volumes for adult liver).

The liver was then finely minced and homogenized using a Brinkmann PT10 polytron; setting 4, pulse time 10 seconds (Brinkmann Inc., Rexdale, Ont.). The homogenate volume was measured and an aliquot (approximately 5 mls for pups and 25 mls for adults) was transferred to a polycarbonate high-speed centrifuge tube. These samples were then placed in ice for up to 30 minutes prior to being centrifuged. Centrifugation at 10,000g, for 30 minutes at 5°C was performed in a Beckman Model J-21 centrifuge equipped with a JA-20 rotor.

An aliquot (3.5 to 4.0 mls - pups; 15 to 20 mls - adults) of the 10,000g supernatant was then removed with a pipette. Four milliliters (adult) or 0.5 ml (pups) of this supernatant was then transferred to a clean 13x100 mm test tube, sealed with parafilm and stored in ice for FDP determinations. The remainder of the supernatant (3 to 3.5 mls - pups; 10 to 15 mls - adults) was then centrifuged at 100,000g for 70 minutes at 5°C in a Beckman Model L5-65 ultracentrifuge equipped with a 60 Ti rotor.

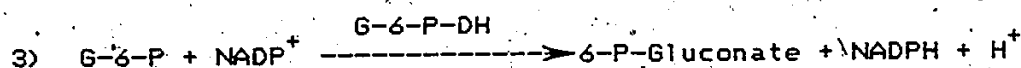
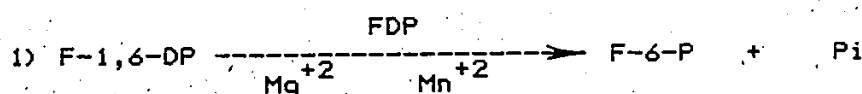
The microsomal pellet was then rinsed twice with ice-cold 0.25 M sucrose, drained and resuspended in 0.25 M sucrose (resuspension volume: 1.0 ml - pups; 4.0 ml - adults). The microsomal suspension was then transferred to a clean 13x100 mm test tube, parafilm sealed and stored in ice for subsequent analysis of G6P and PEPCK activities.

Protein content of the microsomal suspension, and of the 10,000g and 100,000g supernatant was determined by the method of Lowry et al. (1951), using bovine serum albumin as the reference standard.

Dii. Hepatic enzyme studies - quantification.

a) Fructose-1,6-diphosphatase.

Fructose-1,6-diphosphatase activity was assayed according to the method of Latzo & Gibbs (1974). The principle of this method is:



where: F-1,6-DP : Fructose-1,6-diphosphate
 F-6-P : Fructose-6-phosphate
 Pi : inorganic phosphate
 PGI : Phosphoglucose Isomerase
 G-6-P : Glucose-6-phosphate

NADP⁺ : Nucleotide adenine diphosphate - oxidized
 NADPH : Nucleotide adenine diphosphate - reduced
 G-6-P-DH : Glucose-6-phosphate dehydrogenase

The formation of NADPH per unit time, as determined by the increase in absorbance at 340 nm is a measure of FDP activity.

Thus in the assay as performed by us, the ice-cold 10,000g supernatant was diluted (1:10 - pups; 1:20 - adults) with 0.5 M. Tris buffer (pH 7.4). Prior experiments had shown that preparations so diluted would cause an absorbance change that would not exceed 0.100 A/minute. Prior to use, the sample preparation was allowed to stand for 10 minutes at room

temperature (22° C).

The assay mix, (total volume - 1.25 mls) contained:

| | |
|--|------------------|
| Tris-HCl buffer (pH 7.4) | 0.14 M |
| Magnesium Chloride ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$) | 20 mM |
| 2-Mercaptoethanol | 22 mM |
| NADP | 1 mM |
| Fructose-1,6-diphosphate | 1 mM |
| Phosphoglucose Isomerase | 4 U/ml assay mix |
| Glucose-6-phosphate dehydrogenase | 1 U/ml assay mix |
| Sample (diluted preparation) | 0.20 ml |

The increase in absorbance at 340 nm was measured against a reagent blank in which the 10,000g supernatant preparation had been replaced with 0.20 ml of 0.5 M Tris buffer (pH 7.4). The absorbance was recorded every minute for five minutes and the mean AE/minute was calculated.

A reference d-fructose-1,6-diphosphatase, (Sigma Chemical Co.) specific activity of five μmole fructose-1,6-diphosphate converted/minute/mg protein (ie. 5 Units/mg protein at pH 9.5 and 25° C), was used to determine the relationship between absorbance change (AE/minute) and Units under our conditions of pH 7.4 and 22° C.

Several different concentrations of this reference FDP were analysed simultaneously with each set of samples. Unit activities of the samples were determined by comparison to the activity of the reference standards. Sample FDP activity is expressed as Units/g wet liver weight.

b) Glucose-6-phosphatase.

The glucose-6-phosphatase activity in the microsomal fraction was assayed by the method of Baginski et al. (1974). In that method, G6P hydrolyses glucose-6-phosphate to form

glucose and inorganic phosphate (Pi). The liberated phosphate is bound by molybdate (ammonium molybdate) to form a phosphomolybdate complex which is then reduced by ascorbic acid to give a deep blue phosphomolybdous complex which has a peak absorbance at 700 nm. Excess molybdate is bound with an arsenite-citrate solution so that it cannot react with other phosphate esters or with Pi formed by acid hydrolysis of the substrate. The amount of phosphate liberated per unit time, determined as the blue phosphomolybdous complex at 700 nm, is a measure of G6P activity (Baginski et al., 1974).

The method was modified in that EDTA was omitted from the 0.25 M sucrose solution used to resuspend the microsomal pellet. Molar concentrations of the reactants in the assay mix and the assay procedure utilized, were otherwise exactly as those described by Baginski et al. (1974).

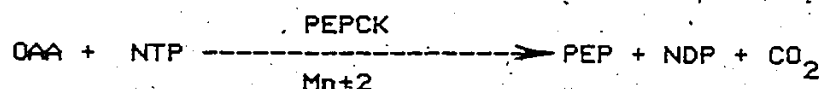
Sample G6P activity was expressed as Units/g wet liver weight, where one Unit of activity will convert one μ mole of glucose-6-phosphate into glucose and Pi/ minute/ mg of microsomal protein at pH 6.5 and 37° C.

Glucose-6-phosphate (Sigma Chemical Co.: specific activity 0.071 Units/ mg protein) was used to test the accuracy of the Baginski method. The activity determined by the Baginsky method was compared to the activity claimed by Sigma. Assayed activity consistently underestimated Sigma stated activity by 11%.

c) Phosphoenolpyruvate carboxykinase.

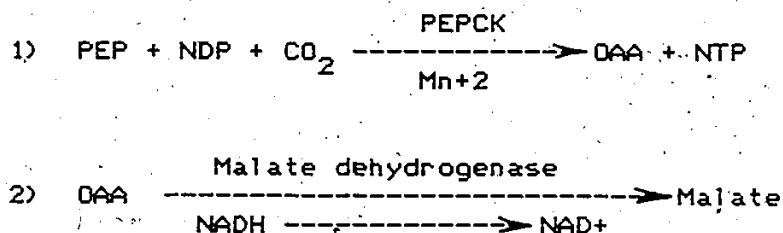
The method of Petrescu et al. (1979) was modified and used to assay PEPCK activity.

The gluconeogenic pathway in which PEPCK acts is:



where; OAA : Oxaloacetic acid
 NTP : guanosine, inosine or xanthosine nucleotide tri-phosphate
 PEP : phosphoenolpyruvate
 NDP : guanosine, inosine or xanthosine nucleotide di-phosphate
 CO₂ : carbon dioxide

In Petrescu's method, PEPCK activity is assayed by reversing the above reaction and coupling it to the formation of malate from OAA and NADH by malate dehydrogenase. Thus the principle reactions are:



The resulting decline in NADH absorbance at 340 nm is measured over time, and PEPCK activity is expressed as $\mu\text{m NADH oxidized/minute}$ at pH 7.5 and 25° C.

The following modifications to Petrescu's method were made.

- 1) The assay was performed at 22° C rather than at 25° C.
- 2) The final volume of the assay mix was 4 ml and not 1 ml.
- 3) GDP (guanosine-5'-diphosphate) replaced dGDP (2'-deoxy-guanosine-5'-diphosphate).

In the rat, hepatic PEPCK activity is usually measured in the cytosolic fraction because PEPCK is primarily distributed

in the cytosol of this animal (Mayes, 1977b). However, cytosolic preparations also contain high concentrations of pyruvate kinase. Often the concentration of this enzyme may exceed that of PEPCK by 5 to 15 fold, (Petrescu et al., 1979). Since GDP is a good substrate for both these enzymes, considerable interference (ie. substrate competition for phosphoenolpyruvate) due to pyruvate kinase is common in PEPCK assays which use cytosol as the enzyme source and GDP as the initiating nucleotide, (Petrescu et al., 1979). Such interference was also seen by us. Thus replicate determinations performed on the same cytosolic sample often yielded inconsistent PEPCK activities. However, no such inconsistency was seen when the microsomal fraction was assayed. Consequently, as a final modification of Petrescu's method, microsomal PEPCK rather than cytosolic PEPCK was quantitated.

Final molar concentrations of reactants in our assay were as follows:

(final volume of mix - 4.0 ml)

| | |
|---|---------------------|
| Tris-HCl buffer (pH 7.4) | 50.00 mM |
| Sodium bicarbonate (sat. with CO ₂) | 20.00 mM |
| Phosphoenolpyruvate | 0.50 mM |
| Manganese chloride | 1.00 mM |
| NADH | 0.15 mM |
| Malate dehydrogenase | 2.5 Units/ml of mix |
| GDP | 0.20 mM |

Sample (0.2 to 1.0 mg microsomal protein/assay) 0.50 ml

In view of the above modifications, our assay was assessed in regards to enzyme kinetics and substrate inhibition.

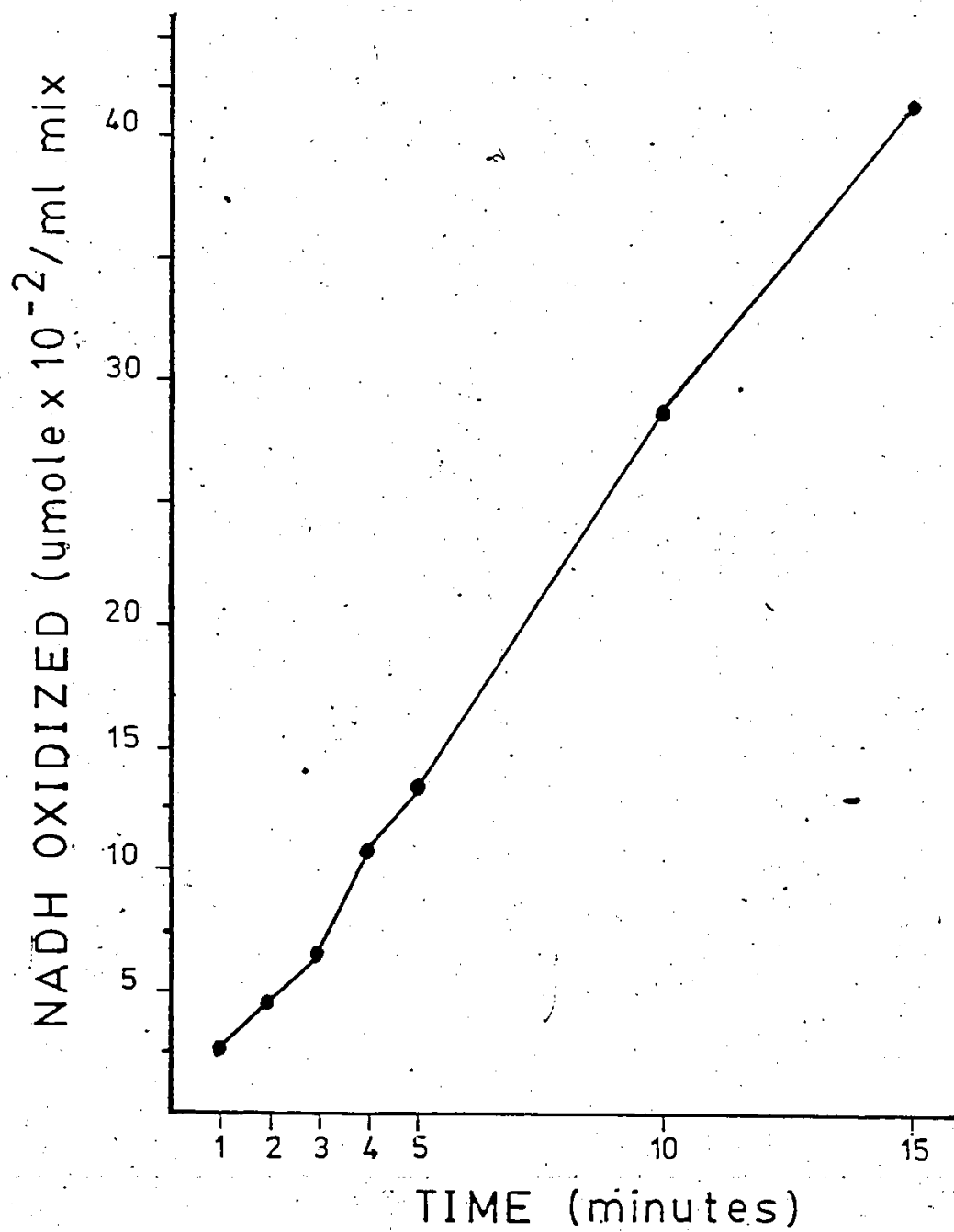
From Figure 1, PEPCK activity versus time, it is apparent that the reaction is linear for 15 minutes. Reaction time was

Figure 1. Phosphoenolpyruvate carboxykinase assay, enzyme kinetics - NADH oxidized ($\mu\text{mole} \times 10^{-2}/\text{ml}$ of assay mix) versus time (minutes). Curve represents mean of 10 samples.

Assay conditions:

substrate concentration - 0.5mM phosphoenolpyruvate
sample concentration - 1.0 mg microsomal protein/ml assay mix

temperature - 22° C
pH - 7.4 units



therefore standardized at 10 minutes. From Figure 2, PEPCK activity versus enzyme concentration, it is clear that enzyme concentrations of 0.25 to 1.0 mg microsomal protein/ml of assay mix produce a linear response in respect to PEPCK activity. Finally, from Figure 3, PEPCK activity versus substrate (phosphoenolpyruvate) concentration, it is apparent that a substrate concentration of 0.25 mM in the assay mix will saturate an enzyme concentration of 1.0 mg microsomal protein/ml. Our assay used a substrate concentration of 0.5 mM in the assay mix.

Unit activity of PEPCK expressed as Units/g of wet liver weight was calculated according to the formulas presented in Bergmeyer et al. (1974). By definition, 1.0 Unit of PEPCK activity will cause the oxidation of 1.0 μ mole of NADH/minute/mg of microsomal protein at pH 7.5 and 22° C.

E. Effects of exogenous d-glucose on plasma glucose and mortality in HEOD-treated male rats.

El. Adult males.

In an attempt to determine if the hyperglycemia in HEOD-treated rats is an adaptive or toxic response, glucose was administered to HEOD-exposed animals.

The rats were fasted and randomly assigned to treatment or control groups. Treatment animals were given HEOD (dose 1 LD50) while controls received corn oil. All rats were then given d-glucose p.o. (6 g/kg as a 60% w/v aqueous solution). Next, a five millimeter piece was removed from the tip of the tail and a mixed arteriovenous blood sample (about 60 μ l)

Figure 2. Phosphoenolpyruvate carboxykinase assay, enzyme

kinetics - NADH oxidized ($\mu\text{mole} \times 10^{-3}/\text{ml}$ of assay mix/minute) versus enzyme concentration (microsomal protein mg/ml in assay mix). Curve represents mean of five replicates.

assay conditions:

substrate concentration - 0.5mM phosphoenolpyruvate
incubation time - 10 minutes

temperature - 22° C
pH - 7.4 Units

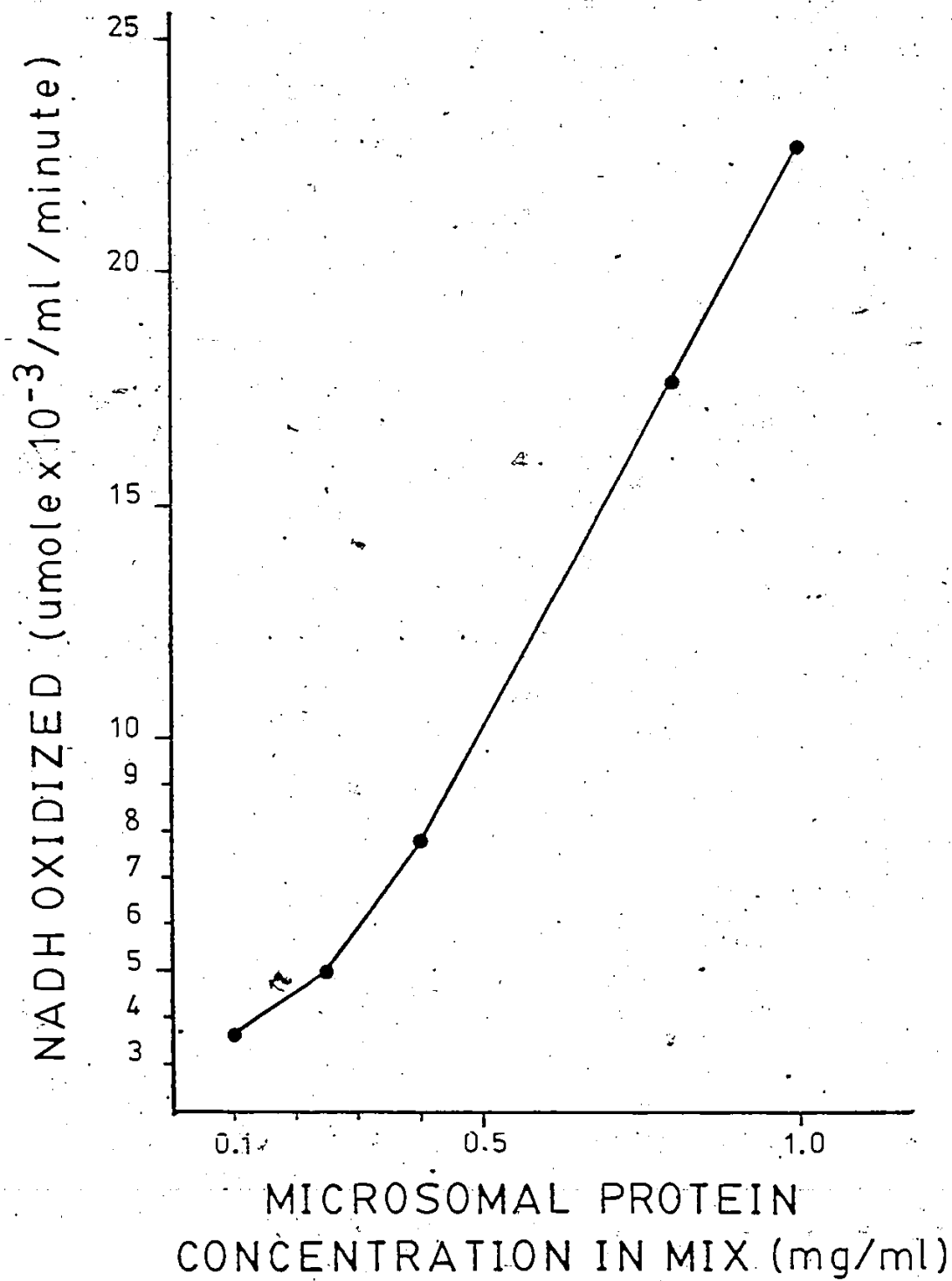
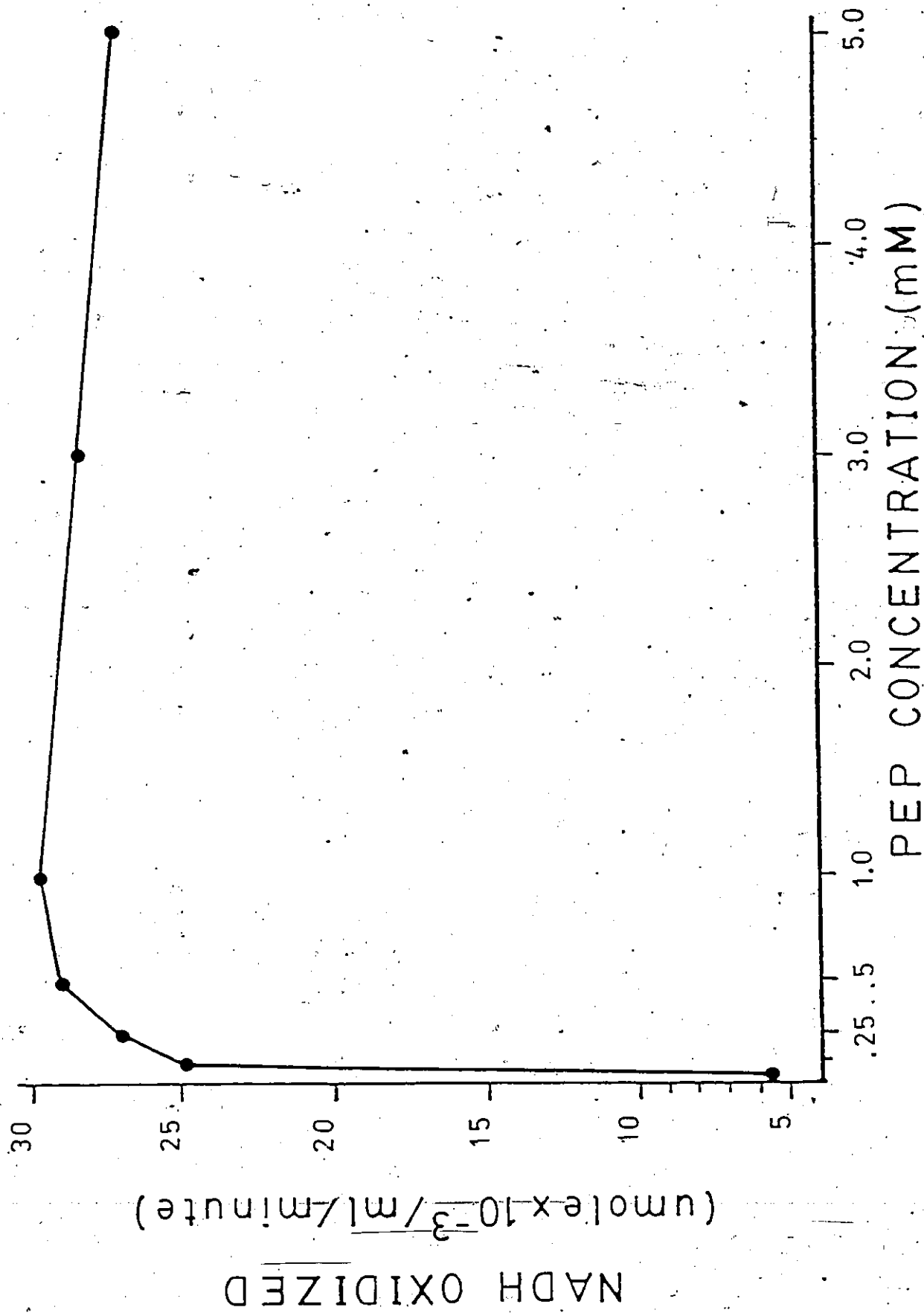


Figure 3. Phosphoenolpyruvate carboxykinase assay, substrate saturation - NADH oxidized ($\mu\text{mole} \times 10^{-3}/\text{ml}$ of assay mix/minute) versus substrate concentration (phosphoenolpyruvate, PEP, mM). Curve represents mean of five samples.

assay conditions:
enzyme concentration - 1.0 mg microsomal protein/ml of assay mix
incubation time - 10 minutes
temperature - 22°C
pH - 7.4 Units



was collected in a capillary tube containing three U.S.P. units of heparin ("Capilets" microhematocrit tubes, Dade Corp., Miami, Fl.).

The blood samples were centrifuged in an I.E.C. Model MB centrifuge (I.E.C., Needham Hts., Mass.) for three minutes at room temperature and 20 μ l of the resulting plasma was withdrawn with a 50 μ l syringe (Hamilton Co., Reno, Nev.). This plasma sample was transferred to a clean 13x100 mm test tube, parafilm sealed and stored frozen at -20° C for plasma glucose analysis.

Blood samples were collected frequently from all rats during the next 24 hours. d-Glucose (6g/kg) was administered every two hours, over this same time. Finally, at the termination of the 24 hour period, the mortality was determined in both the control and treatment groups.

iii. Ten day old pups.

Hyperglycemia was not seen in HEOD-treated 10 day old pups. Therefore, it was of interest to determine what effect on HEOD induced mortality, exogenous glucose might have in these pups.

Ten day old pups (mixed sexes) were fasted and randomly assigned to treatment and control groups. All pups were given HEOD (1 LD₅₀). Treatment pups received d-glucose p.o. (6 g/kg as a 60% aqueous solution) while controls were given an appropriate volume of distilled water.

The pups were then caged individually and placed in an incubator at $28 \pm 1^{\circ}$ C and observed for the next 48 hours.

Pups were fasted throughout this entire period. Exogenous glucose (6g/kg) or distilled water was further administered after 3, 6 and 18 hours. Mortality within the two groups was measured at 24 and 48 hours.

F. Effects of 2-deoxyglucose on: plasma glucose, convulsion onset latency time and mortality in HEOD-treated male rats.

According to Houpt & Hance (1977) the glucose analogue 2-deoxyglucose, as 2-deoxyglucose-6-phosphate, competes intracellularly with glucose-6-phosphate for both phosphohexose isomerase and for hexokinase. This competition results in an impairment of normal cellular glucose metabolism. Additionally, 2-deoxyglucose competes with glucose for the blood-brain-barrier-transport system in the central nervous system. Thus, 2-deoxyglucose causes glucoprivation in the central nervous system. This in turn will increase sympathetic nervous activity and the secretion of adrenal medullary catecholamines which ultimately cause hyperglycemia to develop.

The functional intracellular glucoprivation induced by 2-deoxyglucose treatment might increase the level of mortality seen in the HEOD-treated rat, if HEOD-induced hyperglycemia in such animals is of adaptive value. Consequently, the effect of this analogue in regards to HEOD-induced hyperglycemia and mortality in the adult rat was determined.

Adult males were fasted and assigned to treatment and control groups. All rats were given HEOD (dose 1 LD50). Treatment rats were also given 2-deoxyglucose (1g/kg s.c. as a 48% w/v aqueous solution); controls received an appropriate

volume of distilled water.

Blood samples were taken from the tail at intervals over the 24 hour period following HEOD exposure. In addition to analysis of plasma glucose, plasma 2-deoxyglucose was measured by the method of Crammer and Neville (1953). In that method, 2-deoxyglucose is reacted with "quinaldine reagent" (3,5-diaminobenzoic acid dihydrochloride dissolved in 50% (v/v) phosphoric acid) to produce a yellow coloured product which may then be quantitated by its absorbance at 380 nm.

Samples (50 μ l of plasma) were analysed simultaneously with spiked plasma standards (25 to 300 mg 2-deoxyglucose/dl plasma). Plasma 2-deoxyglucose concentrations, expressed as mg of 2-deoxyglucose/dl plasma, were calculated from a standard curve. Also, the times to first convulsion and the 24 hour mortality observed in both groups were recorded.

G. Effects of insulin on: plasma glucose, hepatic glycogen and PEPCK activity, mortality and convulsion onset latency time in HEOD-treated male rats.

In a further attempt to determine the significance of HEOD-induced hyperglycemia in the adult rat insulin was given to HEOD-treated rats.

Adult rats were fasted, randomly assigned to treatment groups, and given HEOD (1 LD₅₀). The treatment animals were then injected with semi-lente insulin (2 U/kg s.c.) while the controls received an equivalent volume of insulin diluent.¹ Preliminary experiments had shown that a 2 U/kg dose of

1. Appendix 2 gives the composition of insulin diluent.

insulin would cause a 76% decrease in plasma glucose concentration, two hours after administration. This level of hypoglycemia was not fatal in the normal (non-HEOD exposed) adult rat.

Blood samples were taken from the tail at hourly intervals for the next six hours. Some rats from both groups were killed for liver samples two hours after HEOD treatment.

The animals were observed periodically throughout the 24 hours following HEOD treatment so that the time to first convulsion could be measured. Also, the 24 hour mortality was measured in both groups.

H. Effects of phenobarbital on: plasma glucose, hepatic glycogen and PEPCK activity, mortality and convulsion onset latency time in HEOD-treated male rats.

Barbiturates suppress organochlorine pesticide-induced convulsive activity in man (Döull, 1976) and suppress central nervous system-induced seizures in rodents (Wilhjelm & Langgard, 1968). Consequently, the effect of phenobarbital on HEOD responses was measured in the adult rat.

Adult males were fasted and assigned to experimental groups. Treatment animals were given HEOD (dose 1 LD50), while controls received corn oil. All rats were then injected with sodium phenobarbital (40 mg/kg i.p.). The barbiturate was dissolved in water:ethanol:propylene glycol (5:1:4), Krough (1981).

Blood samples were taken from the tail periodically over the next 24 hours. Some rats from both groups were killed to obtain liver samples two hours after HEOD. The animals were

observed during the 24 hour period and the times to first convulsion were recorded. The mortality at 24 hours was also measured for both groups.

I. Effects of phenobarbital and exogenous glucose on: plasma glucose, hepatic glycogen and PEPCK activity, mortality and convulsion onset latency time in HEOD-treated male rats.

Since phenobarbital decreased the plasma glucose and the mortality of HEOD-exposed rats, the effects of phenobarbital were determined in rats given HEOD and glucose.

Adult male rats were fasted and assigned to experimental groups. Treatment animals were given HEOD (dose 1 LD50); controls received corn oil. All rats were then injected with phenobarbital (40 mg/kg i.p.). Immediately following this injection, all rats were given d-glucose (6 g/kg p.o. as a 60% w/v aqueous solution). Supplemental doses of d-glucose were administered 3, 9 and 18 hours later.

Blood samples were taken from the tail at intervals for 24 hours. Some rats from both groups were killed for liver samples two hours after HEOD treatment. The times to first convulsion and the 24 hour mortality in both groups were also recorded.

J. Effects of phenobarbital and insulin on: Plasma glucose, hepatic glycogen and PEPCK activity, mortality and convulsion onset latency time in HEOD-treated male rats.

Insulin increased HEOD-induced mortality and decreased HEOD-induced hyperglycemia. Phenobarbital decreased HEOD-induced mortality and decreased HEOD-induced hyperglycemia.

Consequently, it was of interest to determine if phenobarbital

would alter the effects of insulin in the HEOD-exposed rat.

Adult males were fasted and assigned to treatment and control groups. Treatment animals were given HEOD (dose 1 LD50); controls received corn oil. All rats were then injected with phenobarbital (48 mg/kg i.p.). Immediately following this injection, all rats received semi-lente insulin (2 Units/kg s.c.).

Blood samples were taken from the tail at intervals over the next 24 hours. Some treatment and control rats were killed for liver samples, two hours after HEOD. The times to first convulsion and the 24 hour mortality for both groups were also measured.

K. Effects of atropine on: plasma glucose, hepatic glycogen and PEPCK activity, mortality and convulsion onset latency time in HEOD-treated male rats.

Since atropine prevents vagal stimulation of the heart seen under HEOD intoxication (Gowdy & Stavsky, 1955) the effects of this drug on HEOD-induced mortality and hyperglycemia were measured.

Adult males were fasted as usual and divided into experimental groups. Forty minutes prior to receiving HEOD, treatment group rats were weighed and injected with atropine sulphate (4 mg/kg s.c.), while control group animals received an appropriate volume of 0.9% saline. Forty minutes later (ie, at time zero for the experiment) the animals of both groups were given HEOD (dose 1 LD50). Supplemental doses of atropine or saline were given 4, 8, 12 and 18 hours later.

Blood samples were taken from the tail at intervals over

the next 24 hours. Some animals from both groups were killed for liver samples two hours after HEOD. Measures of the times to first convulsion and the 24 hour mortality for both groups were also made.

L. Effects of L-a-methyldopa on: blood glucose, hepatic glycogen, PEPCK and G6P activities, mortality and convulsion onset latency time in HEOD-treated male rats.

Atropine, a cholinergic blocking agent, decreased the HEOD-induced carbohydrate response in the adult rat. Consequently, it was decided to determine the effects of adrenergic blockade in the HEOD-treated rat. The agent employed was L-a-methyldopa which causes the synthesis of a "false" neurotransmitter in the place of norepinephrine (Weiner, 1980) and which also may act centrally to block sympathetic outflow (Henning, 1969; Weiner, 1980).

Thus, adult male rats, purchased from Woodlyn Laboratories Ltd., were fasted and assigned to experimental groups. All rats were given HEOD (dose 1 LD50). The treatment rats were then injected with L-a-methyldopa (200 mg/kg i.p. administered as a 3% w/v solution in acid saline). Control rats were given an equivalent volume of acid saline. Acid saline is 0.9% saline containing 0.001 M HCl (Uretsky and Seiden, 1969).

A dose of 200 mg/kg was employed since prior experiments showed that it would produce significant reductions in sympathetic nervous activity as evidenced by its sedative effect in HEOD-treated (dose 1 LD50) adult rats. At doses above 200 mg/kg, L-a-methyldopa augmented the toxicity of HEOD. A supplemental dose of L-a-methyldopa or acid saline was given

six hours later.

Blood samples were taken from the tail at intervals over the next 24 hours. Five rats per group were killed at two hours for liver samples for hepatic glycogen, PEPCK and G6P assay. Finally, the times to first convulsion and the 24 hour mortality observed for both groups were recorded.

M. Effects of d-l-propranolol on: plasma glucose, hepatic glycogen and PEPCK activity, mortality and convulsion onset latency time in HEOD-treated male rats.

To further examine the involvement of the sympathetic nervous system in the HEOD-induced carbohydrate response, d-l-propranolol was used to block β -adrenergic receptors.

Adult male rats, purchased from Woodlyn Laboratories Ltd., were fasted as usual. Thirty minutes prior to receiving HEOD (ie. time zero for the experiment) the animals were weighed and assigned to experimental groups. Fifteen minutes prior to time zero, treatment group rats were given d-l-propranolol (8 mg/kg s.c. dissolved in 0.9% saline at a concentration of 0.4% w/v); control group rats received only saline. Finally, at time zero, all animals of both groups received HEOD (dose 1 LD50).

Blood samples were collected from the tails of these rats at intervals over the next 24 hours. Five rats per group were killed at two hours for liver samples. At 5:45 hours after dosing with HEOD, a second d-l-propranolol injection was given to the treatment group rats. Control rats received a second dose of saline at this time. Finally, the time to first convulsion and the 24 hour mortality in both groups were

recorded.

N. Methods of Statistical Analysis.

The data obtained in the above experiments were analysed by standard parametric statistical tests, as described by Rohlf & Sokal (1969), Sokal & Rohlf (1969) and Steel & Torrie (1968). Tests used included, the F-test for homogeneity of variance, analysis of variance (one-way, two-way and two-way paired), Student's t-test (standard comparison of means and the special t-test to compare a single sample variate with a sample mean) and the chi-square test (with and without Yates correction for continuity).

RESULTS

A. Age-specific LD50 for dieldrin.

Table I presents the LD50 for dieldrin in five and ten day old rats (mixed sexes) and in 60 day old adult males.

Table I. Age-specific dieldrin LD50 values (per os) in five and ten day old rats (mixed sexes) and in 60 day old adult males.

| Age (days) | N | LD50 (mg/kg) | 95% confidence limits lower | upper |
|---------------|-----|-----------------|--------------------------------|-------|
| 5 | 151 | 38.0 | 27.2 | 49.6 |
| 10 | 183 | 26.8 | 23.0 | 31.2 |
| 60 | 56 | 63.0 | 55.0 | 72.1 |

N : total number of animals used for determination.

It is clear that the ten day old pup is the most sensitive to HEOD, while the 60 day old adult male is the least sensitive. Furthermore, the LD50 values for all three ages lie outside the 95% confidence limits of every other age and thus are significantly different.

B. Effect of dieldrin on energy substrates.

Bi. Five day old pups.

Table II presents the plasma glucose, plasma FFA and hepatic glycogen levels found in five day old pups from five litters, one and three hours after treatment with HEOD (dose 1 LD50). The table also shows the results of analyses of

Table II: Plasma glucose, plasma FFA and hepatic glycogen in five day old pups, one and three hours after 1 LD50 HEOD. Tabled values represent mean \pm SE of five litters.

| Substrate parameter | Hours post HEOD | CONTROL | | TREATED | | 2-way paired anova | |
|--------------------------|-----------------|-----------------|--|------------------|--|--------------------|----------|
| | | Mean \pm SE | | Mean \pm SE | | Litter F | Treat. F |
| Plasma Glucose (mg/dl) | 1.0 | 63.6 \pm 3.23 | | 74.5 \pm 4.12 | | 8.179a | 19.713a |
| | 3.0 | 57.9 \pm 8.56 | | 52.8 \pm 10.50 | | 5.186 | 0.434 |
| Plasma FFA (mg/dl) | 1.0 | 26.3 \pm 1.28 | | 23.9 \pm 1.42 | | 22.405b | 17.658a |
| | 3.0 | 17.0 \pm 0.74 | | 17.1 \pm 1.86 | | 4.410 | 0.003 |
| Hepatic glycogen (ug/mg) | 1.0 | 6.5 \pm 0.94 | | 5.1 \pm 0.68 | | 21.009b | 18.274a |
| | 3.0 | 9.1 \pm 2.28 | | 6.0 \pm 1.26 | | 3.32 | 3.18 |

control group received only corn oil vehicle

Treat. : treatment sub-group (HEOD)

a : $P < 0.05$

b : $P < 0.01$

variance (2-way paired anova) performed on the data.

Dieldrin one hour after administration elevated plasma glucose and decreased both plasma FFA and hepatic glycogen concentrations. These changes were transitory however and, by three hours after treatment, there were no differences in the substrates between control and treated groups.

Significant differences between litters were also seen one hour after HEOD. Such "litter" effects were expected since the pups of the different litters had a different genetic and environmental heritage, having been born of, and raised by, different dams.

Bii. Ten day old pups.

In view of the sensitivity to HEOD exhibited by the ten day old pup, two doses of HEOD ($1/2$ and 1 LD₅₀) were used to assess the compounds' effects on substrates in that age group.

Dieldrin at a dose of $1/2$ LD₅₀ did not significantly alter any of the substrate concentrations either one or three hours after administration (Table III). Furthermore, even when the dose was increased to one LD₅₀, the levels of plasma glucose and FFA were unchanged both at one and three hours (Table IV). However the higher dose did significantly reduce the hepatic glycogen levels (16%). Additionally, as in the case of the five day old pup, this effect was also transitory since equivalent glycogen levels were found in control and treated pups at three hours (Table IV).

These results indicate that the greater toxicity of HEOD to the 10 day old pup does not result from reduced availability

Table III. Plasma glucose, plasma FFA and hepatic glycogen in ten day old pups, one and three hours after 1/2 LD50 HEOD. Tabled values represent mean \pm SE of five and six litters, at one hour and three hours respectively post HEOD.

| Substrate parameter | Hours post HEOD | CONTROL Mean \pm SE | TREATED Mean \pm SE | 2-way paired anova | |
|--------------------------|-----------------|--------------------------|--------------------------|--------------------|-------------|
| | | | | Litter F | Treat. F |
| Plasma glucose (mg/dl) | 1.0 | 78.6 \pm 7.22 | 83.8 \pm 5.87 | 4.027 | 0.215 |
| | 3.0 | 62.8 \pm 2.45 | 62.9 \pm 3.81 | 3.225 | 0.0003 |
| Plasma FFA (mg/dl) | 1.0 | 25.9 \pm 0.83 | 24.2 \pm 0.87 | 0.346 | 1.351 |
| | 3.0 | 20.4 \pm 1.74 | 27.2 \pm 5.29 | 3.270 | 2.554 |
| Hepatic glycogen (ug/mg) | 1.0 | 9.8 \pm 3.23 | 8.8 \pm 2.53 | 15.035a | 0.524 |
| | 3.0 | 3.3 \pm 1.40 | 3.3 \pm 1.63 | 42.169b | 0.0008 |

control group received only corn oil vehicle

Treat. : treatment sub-group (HEOD)

a : $P < 0.05$

b : $P < 0.01$

Table IV. Plasma glucose, plasma FFA and hepatic glycogen in ten day old pups, one and three hours after 1 LD50 HEOD. Tabled values represent mean \pm SE of five litters.

| Substrate parameter | Hours post HEOD | CONTROL | | TREATED | | 2-way paired anova | |
|--------------------------|-----------------|-----------------|--|-----------------|--|--------------------|----------|
| | | Mean \pm SE | | Mean \pm SE | | Litter F | Treat. F |
| Plasma glucose (mg/dl) | 1.0 | 76.0 \pm 6.51 | | 75.6 \pm 6.29 | | 34.530b | 8.035 |
| | 3.0 | 75.8 \pm 3.02 | | 83.5 \pm 5.36 | | 2.306 | 2.619 |
| Plasma FFA (mg/dl) | 1.0 | 32.8 \pm 2.30 | | 30.3 \pm 1.69 | | 2.515 | 1.317 |
| | 3.0 | 22.5 \pm 2.24 | | 27.0 \pm 3.69 | | 3.455 | 2.500 |
| Hepatic glycogen (ug/mg) | 1.0 | 17.4 \pm 2.91 | | 14.6 \pm 2.41 | | 30.720b | 8.706a |
| | 3.0 | 5.5 \pm 0.20 | | 4.2 \pm 1.45 | | 1.027 | 1.386 |

control group received only corn oil vehicle

Treat. : treatment sub-group (HEOD)

a : $P < 0.05$

b : $P < 0.01$

of energy substrates. Furthermore, it is clear that HEOD does not cause a significant HEOD-induced hyperglycemic response in the ten day old rat. Significant litter effects were seen in these animals (Tables III and IV) and probably reflect the variable nutritional states of the pups within the different litters.

Biii. Sixty day old adult males.

Table V presents the plasma glucose, plasma FFA and hepatic glycogen levels in 60 day old male rats, one and three hours after treatment with HEOD (1 LD50).

It is apparent that HEOD significantly increased the plasma glucose by 45% and 30% one and three hours after treatment, respectively. However, the pesticide had no effect on plasma FFA or on hepatic glycogen at either of these times. Thus dieldrin will evoke a significant hyperglycemic response in the adult male rat.

In view of the ability of HEOD to cause hyperglycemia in the 60 day old rat, it was of interest to determine if it could also induce such a response in older rats. Consequently 120 and 200 day old male rats were given HEOD (dose: 1/2 or 1 60 day old age-specific LD50) and the plasma glucose, plasma FFA and hepatic glycogen levels were measured (Table VI).

Dieldrin, under all conditions, was effective in inducing hyperglycemia in both the 120 and 200 day old males. In fact the relative increases in plasma glucose levels, compared to controls values, were 1.5 fold greater than those found in the 60 day old rat (Table VI).

Table V. Plasma glucose, plasma FFA and hepatic glycogen in 60 day old male rats, one and three hours after 1 LD50 HEOD. Tabled values represent mean \pm SE of N individuals.

| Substrate parameter | Hours post HEOD | N | CONTROL Mean \pm SE | N | TREATED Mean \pm SE | One-way anova F |
|--------------------------|-----------------|---|-----------------------|----|-----------------------|-----------------|
| Plasma glucose (mg/dl) | 1.0 | 7 | 92.7 \pm 6.83 | 9 | 134.2 \pm 6.88 | 21.811c |
| | 3.0 | 9 | 96.9 \pm 3.97 | 13 | 126.0 \pm 7.95 | 8.389b |
| Plasma FFA (mg/dl) | 1.0 | 9 | 29.8 \pm 2.94 | 13 | 31.8 \pm 2.77 | 0.243 |
| | 3.0 | 9 | 26.0 \pm 3.33 | 12 | 28.0 \pm 1.45 | 0.362 |
| Hepatic glycogen (ug/mg) | 1.0 | 9 | 16.8 \pm 5.17 | 13 | 14.9 \pm 3.18 | 0.127 |
| | 3.0 | 9 | 6.7 \pm 3.32 | 12 | 8.5 \pm 3.46 | 0.218 |

control group received only corn oil vehicle

b : $P < 0.01$

c : $P < 0.001$

Table VI. Percent increases in treated versus control mean plasma glucose concentrations found for: 60, 120 and 200 day old male rats at various times after 1/2 or 1, 60 day old age-specific LD50 HEOD (i.e. 31.5 or 63.0 mg/kg).

| Age days | Dose LD50 | Hours post HEOD | N | CONTROL Mean \pm SE | N | TREATED Mean \pm SE | Percent increase |
|----------|-----------|-----------------|---|-----------------------|----|-----------------------|------------------|
| 60 | 1.0 | 1.0 | 7 | 92.7 \pm 6.83 | 9 | 134.2 \pm 6.00 | 44.8c |
| 60 | 1.0 | 3.0 | 9 | 96.9 \pm 3.97 | 13 | 126.0 \pm 7.95 | 30.0b |
| 120 | 0.5 | 1.5 | 5 | 113.4 \pm 3.03 | 5 | 178.4 \pm 15.61 | 57.3b |
| 120 | 1.0 | 1.0 | 6 | 100.0 \pm 3.62 | 6 | 157.8 \pm 6.84 | 57.8c |
| 200 | 0.5 | 1.5 | 3 | 88.2 \pm 3.04 | 5 | 187.3 \pm 24.74 | 112.4a |

control group received only corn oil vehicle.
percent increase (i.e. difference treated versus control) data was analysed by t-test.

a : $P < 0.05$

b : $P < 0.01$

c : $P < 0.001$

In addition, Table VII shows that the higher dose of HEOD (i.e. 1 LD50) produced hyperlipidemia one hour after administration to the 120 day old rat. However, the lower dose (i.e. 1/2 LD50) did not affect plasma lipids in either the 120 or 200 day old rats. The hepatic glycogen levels were unaltered in any of the animals. The adult rats displayed the most pronounced and prolonged hyperglycemia from HEOD exposure. The five day old pup also showed a hyperglycemic response one hour after treatment with HEOD. The ten day old pups, on the other hand, remained euglycemic at both times after HEOD. These results closely parallel the acute toxicity of HEOD in the various age groups. Thus the ages most resistant to HEOD (i.e. the adult and the five day old) also exhibit the greatest degree of hyperglycemia. Conversely, the age group most sensitive to HEOD (i.e. the ten day old) did not show a hyperglycemic response. Therefore, it appears that the hyperglycemia is an adaptive response in the resistant animals.

C. Effects of dieldrin on the activities of hepatic gluconeogenic enzymes.

Ci. Plasma glucose.

Table VIII presents the plasma glucose concentrations of five and ten day old pups (mixed sexes) and 60 day old males, one and three hours after treatment with one age-specific LD50 of HEOD. These data were collected as part of a study to determine the effect of HEOD on hepatic gluconeogenic enzymes.

From Table VIII, it is clear that HEOD did not induce

Table VII: Plasma glucose, plasma FFA and hepatic glycogen in 120 and 200 day-old male rats, one or one and one-half hours after HEOD (dose: 1/2 or 1, 60-day old age-specific LD50 - i.e. 31.5 or 63.0 mg/kg). Tabled values represent mean \pm SE of N rats.

| Substrate parameter | Hours post HEOD | N | CONTROL Mean \pm SE | N | TREATED Mean \pm SE | One-way anova F |
|------------------------------|-----------------|---|-----------------------|---|-----------------------|-----------------|
| A. 120 day old 1/2 LD50 : | | | | | | |
| Glucose | 1.5 | 5 | 113.4 \pm 3.03 | 5 | 178.4 \pm 15.61 | 16.733b |
| FFA | 1.5 | 5 | 24.5 \pm 3.71 | 6 | 32.4 \pm 3.66 | 2.318 |
| Glycogen | 1.5 | 5 | 11.2 \pm 3.64 | 5 | 6.2 \pm 1.02 | 1.733 |
| B. 120 day old 1 LD50 : | | | | | | |
| Glucose | 1.0 | 6 | 100.0 \pm 3.62 | 6 | 157.8 \pm 6.84 | 55.724c |
| FFA | 1.0 | 6 | 26.8 \pm 1.47 | 6 | 34.4 \pm 2.47 | 6.947a |
| Glycogen | 1.0 | 6 | 13.3 \pm 3.62 | 6 | 14.8 \pm 3.75 | 0.106 |
| C. 200 day old 1/2 LD50 : | | | | | | |
| Glucose | 1.5 | 3 | 88.2 \pm 3.04 | 3 | 187.3 \pm 24.74 | 8.986a |
| FFA | 1.5 | 3 | 24.4 \pm 4.23 | 3 | 22.8 \pm 2.27 | 0.125 |
| Glycogen | 1.5 | 3 | 10.4 \pm 2.57 | 3 | 5.6 \pm 1.27 | 4.551 |

control group received only corn oil vehicle

Glucose : plasma glucose (mg/dl)

FFA : plasma FFA (mg/dl) -

Glycogen : hepatic glycogen (ug/mg wet liver weight)

a : $P < 0.05$

b : $P < 0.01$

c : $P < 0.001$

Table VIII. Plasma glucose concentrations (mg/dl) in five and ten day old pups (mixed sexes) and 60 day old male rats, one and three hours after HEOD (1 age-specific LD50). Tabled values represent mean \pm SE.

| Age days | Hours post HEOD | CONTROL | | TREATED | | 2-way paired anova | |
|-------------|-----------------------|------------------|--|-------------------|--|--------------------|-------------|
| | | Mean \pm SE | | Mean \pm SE | | Litter F | Treat. F |
| 5 | 1.0 | 79.7 \pm 3.46 | | 84.3 \pm 2.44 | | 0.490 | 0.867 |
| | 3.0 | 70.8 \pm 2.21 | | 51.4 \pm 13.40 | | 1.135 | 2.160 |
| 10. | 1.0 | 90.6 \pm 7.27 | | 101.6 \pm 11.46 | | 10.801a | 3.861 |
| | 3.0 | 75.7 \pm 10.16 | | 78.0 \pm 26.55 | | 6.335 | 0.079 |
| | | | | | | One-way anova | |
| | | | | | | F | |
| 60 | 1.0 | 95.9 \pm 4.31 | | 133.6 \pm 10.21 | | | 11.564b |
| | 3.0 | 87.3 \pm 4.22 | | 125.9 \pm 10.83 | | | 12.179b |

control group received only corn oil vehicle

treat. : treatment sub-group (HEOD).

sample sizes : for five and ten day old - 10 pups (5 litters)

for 60 day old males - 8 rats

a : $P < 0.05$

b : $P < 0.01$

hyperglycemia in the five or ten day old rat, but it did, one and three hours after treatment, in the 60 day old male rat. These results agree with those previously found for the 60 day old rat and the ten day old pup, but they disagree with those found at one hour in the five day old pup. Consequently, the plasma glucose concentrations in rats of identical age, at identical times after HEOD treatment, in the two studies were compared statistically. The results of these comparisons appear in Tables IX and X.

With the exception of the five day old controls sampled at one hour after treatment, the plasma glucose levels were found to be statistically equivalent between the two studies for rats of identical ages, sampled at identical times after HEOD. This indicates that the effects of HEOD on plasma glucose were constant and reproducible.

The plasma glucose concentrations in the five day old control pups differed by 25% between the two experiments. The reason for this is unknown. However, the plasma glucose levels in the HEOD-treated five day old pups were equivalent at one hour in the two studies. Perhaps therefore, the observed 25% elevation in plasma glucose concentration in the control pups of the second experiment is masking an otherwise significant HEOD induced hyperglycemic response.

Cii. Hepatic fructose-1,6-diphosphatase activity.

Hepatic FDP activities were measured in five and ten day old pups and 60 day old males, one and three hours after treatment with one age-specific LD50 of HEOD (Table XI).

Table IX. Plasma glucose concentration (mg/dl) in control animals of substrate and enzyme studies. Data was collected for five and ten day old pups (mixed sexes) and 60 day old male rats, one and three hours after corn oil vehicle.

| Age days | Hours post oil | SUBSTRATE STUDY | | ENZYME STUDY | | t |
|-------------|----------------------|-----------------|-----------------|--------------|------------------|--------|
| | | N | Mean \pm SE | N | Mean \pm SE | |
| 5 | 1.0 | 5 * | 63.6 \pm 3.23 | 5 * | 79.7 \pm 3.46 | 3.399b |
| | 3.0 | 5 * | 57.9 \pm 8.56 | 5 * | 70.8 \pm 2.21 | 1.460 |
| 10 | 1.0 | 5 * | 76.0 \pm 6.51 | 5 * | 90.6 \pm 7.27 | 1.496 |
| | 3.0 | 5 * | 75.8 \pm 3.02 | 5 * | 75.7 \pm 10.16 | 0.009 |
| 60 | 1.0 | 7 ** | 92.7 \pm 6.83 | 8 ** | 95.7 \pm 4.31 | 0.379 |
| | 3.0 | 9 ** | 96.9 \pm 3.97 | 8 ** | 87.3 \pm 4.22 | 1.656 |

* : litters

** : individuals not litters

b : $P < 0.01$

Table X. Plasma glucose concentration (mg/dl) in HEOD-treated animals of substrate and enzyme studies. Data was collected for five and ten day old pups (mixed sexes) and 60 day old male rats, one and three hours after HEOD (1 age-specific LD50).

| Age days | Hours post HEOD | SUBSTRATE STUDY | | ENZYME STUDY | | t |
|-------------|-----------------------|-----------------|------------------|--------------|-------------------|-------|
| | | N | Mean \pm SE | N | Mean \pm SE | |
| 5 | 1.0 | 5 * | 74.5 \pm 4.12 | 5 * | 84.3 \pm 2.44 | 2.045 |
| | 3.0 | 5 * | 52.8 \pm 10.50 | 5 * | 51.4 \pm 13.50 | 0.082 |
| 10 | 1.0 | 5 * | 75.6 \pm 6.29 | 5 * | 101.6 \pm 11.46 | 1.969 |
| | 3.0 | 5 * | 83.5 \pm 5.36 | 5 * | 78.0 \pm 26.55 | 0.422 |
| 60 | 1.0 | 9 ** | 134.2 \pm 6.00 | 8 ** | 133.6 \pm 10.21 | 0.044 |
| | 3.0 | 13 ** | 126.0 \pm 7.95 | 8 ** | 125.9 \pm 10.83 | 0.008 |

* : litters

** : individuals not litters.

Table XI. Hepatic fructose-1,6-diphosphatase activity (U/g wet liver weight) in five and ten day old pups (mixed sexes) and 60-day old male rats, one and three hours after HEOD (1 age-specific LD50). Tabled values represent mean \pm SE.

| Age days | Hours post HEOD | CONTROL Mean \pm SE | TREATED Mean \pm SE | 2-way paired anova | |
|---------------|-----------------|--------------------------|--------------------------|--------------------|-------------|
| | | | | Litter F | Treat. F |
| 5 | 1.0 | 5.81 \pm 0.491 | 6.77 \pm 0.671 | 14.154a | 9.983a |
| | 3.0 | 4.53 \pm 0.573 | 3.96 \pm 0.309 | 0.922 | 0.736 |
| 10 | 1.0 | 8.99 \pm 1.642 | 8.86 \pm 1.345 | 2.924 | 0.007 |
| | 3.0 | 5.49 \pm 0.600 | 4.96 \pm 0.584 | 44.545b | 9.059a |
| One-way anova | | | | | |
| F | | | | | |
| 60 | 1.0 | 3.60 \pm 0.196 | 4.12 \pm 0.247 | | 2.661 |
| | 3.0 | 3.47 \pm 0.187 | 4.31 \pm 0.655 | | 1.714 |

control group received only corn oil vehicle

treat. : treatment sub-group (HEOD)

sample sizes : for five and ten day old - 10 pups (5 litters)
for 60 day old males - 8 rats

a : $P < 0.05$

b : $P < 0.01$

The FDP activities found in control animals agree with values cited by Ballard & Oliver (1962) for young rats and with those cited by Underwood & Newsholme (1965) and Bhatia et al. (1973) for adult rats.

Dieldrin significantly elevated the activity of FDP by 16.5% one hour after administration in the five day old pup and decreased FDP activity by 10% in the 10 day old pup at three hours. At all other ages and times, HEOD did not alter hepatic FDP activity.

Ciii. Hepatic glucose-6-phosphatase activity.

Hepatic G6P activities were measured in five and ten day old pups and 60 day old males, one and three hours after treatment with one age-specific LD50 of HEOD (Table XII).

The G6P activities found in control animals agree with those cited by Greengard (1969) for young rats and with those cited by Baginski et al. (1967), Platt & Cockrell (1969) and Herrman & Nordlie (1972) for adult rats.

Dieldrin was found to have no effect on G6P activity and therefore it is unlikely that this enzyme plays a significant role in the elicitation of the HEOD-induced carbohydrate response.

Civ. Hepatic phosphoenolpyruvate carboxykinase activity.

Table XIII presents the hepatic PEPCK activities found in five and ten day old pups (mixed sexes) and 60 day old males, one and three hours after administration of one age-specific LD50 of HEOD.

Table XII. Hepatic glucose-6-phosphatase activity (U/g wet liver weight) in five and ten day old pups (mixed sexes) and 60 day old male rats, one and three hours after HEOD (1 age-specific LD50). Tabled values represent mean \pm SE.

| Age days | Hours post HEOD | CONTROL Mean \pm SE | TREATED Mean \pm SE | 2-way paired anova | |
|-------------|-----------------------|--------------------------|--------------------------|--------------------|-------------|
| | | | | Litter F | Treat. F |
| 5 | 1.0 | 3.95 \pm 0.544 | 4.81 \pm 0.275 | 1.751 | 2.727 |
| | 3.0 | 4.04 \pm 0.400 | 5.96 \pm 1.069 | 1.780 | 3.942 |
| 10 | 1.0 | 4.15 \pm 0.743 | 6.18 \pm 0.869 | 1.233 | 3.523 |
| | 3.0 | 4.71 \pm 0.541 | 6.96 \pm 1.635 | 3.056 | 3.462 |
| ----- | | | | | |
| | | | | One-way anova | |
| | | | | F | |
| 60 | 1.0 | 10.75 \pm 0.804 | 11.40 \pm 0.630 | | 0.410 |
| | 3.0 | 8.72 \pm 1.100 | 10.06 \pm 1.381 | | 0.593 |

control group received only corn oil vehicle

treat. : treatment sub-group (HEOD)

sample sizes : for five and ten day old - 10 pups (5 litters)

for 60 day old males - 8 rats

Table XIII. Hepatic phosphoenolpyruvate carboxykinase activity (U/g wet liver weight) in five and ten day old pups and 60 day old male rats, one and three hours after HEOD (1 age-specific LD50). Tabled values represent mean \pm SE.

| Age days | Hours post HEOD | CONTROL | | TREATED | | 2-way paired anova | |
|-------------|-----------------------|-------------------|--|-------------------|--|--------------------|---------------|
| | | Mean \pm SE | | Mean \pm SE | | Litter F | Treat. F |
| 5 | 1.0 | 0.060 \pm 0.009 | | 0.077 \pm 0.010 | | 0.671 | 1.308 |
| | 3.0 | 0.221 \pm 0.029 | | 0.389 \pm 0.045 | | 2.139 | 15.565a |
| 10 | 1.0 | 0.202 \pm 0.057 | | 0.214 \pm 0.060 | | 4.828 | 0.172 |
| | 3.0 | 0.220 \pm 0.032 | | 0.474 \pm 0.086 | | 2.314 | 12.708a |
| | | | | | | | One-way anova |
| | | | | | | | F |
| 60 | 1.0 | 1.150 \pm 0.112 | | 1.490 \pm 0.105 | | | 4.912a |
| | 3.0 | 0.846 \pm 0.063 | | 1.321 \pm 0.125 | | | 12.469b |

control group received only corn oil vehicle

treat. : treatment sub-group (HEOD)

sample sizes : for five and ten day old - 10 pups (5 litters)

for 60 day old males - 8 rats

a : $P < 0.05$

b : $P < 0.01$

It is apparent that HEOD increased hepatic PEPCK activity, three hours after administration to immature and adult rats. In addition, the activity of this enzyme was also increased, one hour after HEOD treatment in the adult rat.

iv. Relevance of enzyme study results.

Phosphoenolpyruvate carboxykinase, glucose-6-phosphatase and fructose-1,6-diphosphatase are three of the four rate-limiting gluconeogenic enzymes of the liver. Thus, the above data suggest that the hyperglycemia found in the HEOD-exposed adult rat may result from increased gluconeogenesis.

Consistent, reproducible hyperglycemia both at one and three hours after HEOD was exhibited only by the 60 day old rat. Animals of this age were also the only ones with elevated PEPCK activities at both one and three hours. PEPCK facilitates the net transfer of oxaloacetic acid out of the tricarboxylic acid cycle and into the main pathway of gluconeogenesis. Through this function, PEPCK effectively controls the overall rate of influx of non-glycerol gluconeogenic precursors into the anabolic pathway and is therefore generally considered to be the major rate-limiting enzyme of gluconeogenesis (Mayes, 1977a). Therefore, it is concluded that the HEOD-induced hyperglycemia is caused by PEPCK-stimulated gluconeogenesis in the adult rat.

Elevated PEPCK activity, three hours after HEOD was also seen in the immature animals. However no hyperglycemia was found in these pups, three hours after HEOD. Why did the enhanced PEPCK activity not elicit a hyperglycemic response?

In the case of the ten day old, the lack of gluconeogenesis may have resulted from the 10% decrease in FDP activity. Thus the decreased FDP may have counteracted the increased PEPCK activity to produce an insufficient level of gluconeogenesis to allow hyperglycemia to develop.

The situation in the five day old pup is considerably more confusing. One hour after the administration of HEOD, FDP activity was elevated by 17%, but PEPCK activity was unaltered. Furthermore, reproducible hyperglycemia at one hour was not demonstrable in the five day old. These results indicate that while the five day old pup may have the capacity to produce a hyperglycemic response one hour after HEOD, this response does not result solely from enhanced FDP activity.

Dieldrin three hours after administration to the five day old pup increased PEPCK activity by 77%. However this enhanced PEPCK activity was not accompanied by any significant hyperglycemia. Why hyperglycemia was not produced despite increased hepatic PEPCK activity is not known. In conclusion, it is clear that in the immature rat, HEOD may increase the activity of one or more of the key gluconeogenic enzymes, but this does not necessarily elicit a corresponding hyperglycemic response.

D. Exogenous d-glucose: effects in HEOD-treated rats.

Di. Adult males.

In the adult rat, resistance to the acute toxicity of HEOD appeared to be inversely correlated to the ability of the pesticide to induce hyperglycemia. Consequently, 60 day old male rats were treated once with HEOD (1 LD₅₀) and then every

two hours with d-glucose (6 g/kg p.o.) for 24 hours. Figure 4 presents the plasma glucose concentrations and the 24 hour mortality observed in these animals.

From Figure 4, it is apparent that in rats given either corn oil or HEOD, the administration of glucose causes a marked elevation of plasma glucose. However, the glucose concentrations in the HEOD-treated rats were considerably greater than those in the control animals. For example, the increase at 24 hours in HEOD-treated rats was 350%.

Figure 4 also shows that exogenous glucose increased the 24 hour mortality of the HEOD-treated rats by 66% when compared to the mortality expected on the basis of treatment with HEOD alone (i.e. 1 LD50, 58% mortality). However, this increase in mortality was not statistically significant ($\chi^2 = 1.69$).

The plasma glucose data (Figure 4) was subjected to a 2-way anova (Table XIV).

Table XIV. Results of 2-way anova on mean plasma glucose concentrations observed in 60 day old male rats given HEOD (1 LD50) and d-glucose (6 g/kg p.o.) every two hours for 24 hours.

| Source of variation | df | Mean square | F | Significance |
|---------------------|----|-------------|--------|--------------|
| treatments | 1 | 388895.46 | 19.801 | $P < 0.001$ |
| time | 18 | 33434.35 | 1.702 | $P > 0.05$ |
| error | 18 | 19640.56 | | |
| total | 37 | | | |

df : degrees of freedom

Figure 4. Mean plasma glucose (mg/dl) in 60 day old male rats given either 1 LD50 HEOD (treated) or corn oil (control) and d-glucose (6 g/kg p.o.) every two hours for 24 hours. The figure also presents the 24 hour percent mortality in the treated group.

n: 3 rats (control)

n: 12 rats (treated)

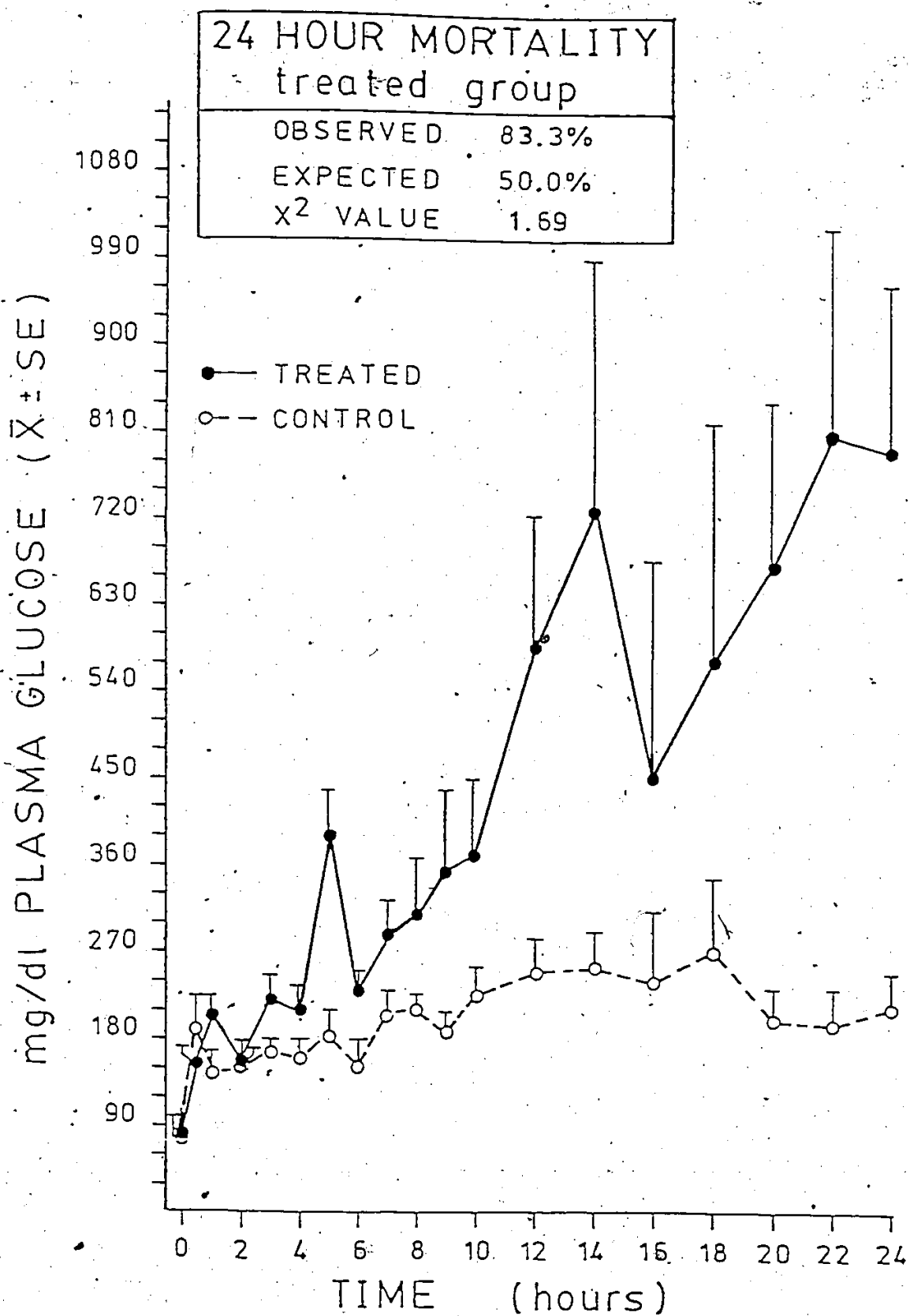


Table XIV shows that the plasma glucose levels of the rats treated with HEOD and glucose were highly significantly different from those in rats given corn oil and exogenous glucose. Thus, the differences in the plasma glucose levels was due to HEOD. Also in both HEOD-treated and control animals, given exogenous glucose, an equivalent trend towards elevated plasma glucose concentrations was evident throughout the 24 hours.

These results suggest that increased hyperglycemia did not confer any protection against the toxicity of dieldrin. No statistically significant change in the 24 hour mortality was demonstrable in the HEOD and glucose-treated rats of this experiment.

Dii. Ten day old pups.

The ten day old pup is the most sensitive to the acute toxicity of HEOD. In addition, HEOD does not cause a hyperglycemic response at this age. Consequently, we determined the effect of d-glucose on the mortality of the HEOD-treated ten day old pup.

Table XV presents the percent mortality, at 24 and 48 hours, of ten day old pups (mixed sexes) treated with 1 LD50 HEOD and d-glucose (6 g/kg p.o.) at 0, 3, 6 and 18 hours after HEOD. Controls received 1 LD50 HEOD and distilled water at the above times.

It is apparent from Table XV that the 24 hour mortality in the control group was equal to that expected on the basis of a 1 LD50 dose of HEOD. In contrast, the 24 hour mortality

Table XV. Mortality at 24 and 48 hours in ten day old pups (mixed sexes) treated with 1 LD50 HEOD and either, 6 g/kg p.o. d-glucose (treated) or distilled water (control) at: 0, 3, 6 and 18 hours after HEOD.

| Time hours | Group | N pups dead | Observed mortality as percent | χ^2 |
|------------|---------|-------------|-------------------------------|---------------------|
| 24 | control | 14/30 | 46.7 | 0.07 ¹ |
| | treated | 3/28 | 10.7 | 10.22b ² |
| 48 | control | 16/30 | 53.3 | 9.04b ³ |
| | treated | 9/28 | 32.1 | 2.65 ⁴ |

- 1: 24 hour mortality of control group versus that expected for a 1 LD50 dose of HEOD
 2: 24 hour mortality of treated group versus that expected for a 1 LD50 dose of HEOD
 3: 24 hour mortality of treated group versus 24 hour mortality of control group
 4: 48 hour mortality of treated group versus 48 hour mortality of control group
 b: $P < 0.01$

suffered by the treated group (i.e. HEOD and glucose group) was significantly less than would have been expected after a 1 LD50 dose of HEOD, and was also significantly less than that suffered by the controls. These results indicate that exogenous glucose reduced the acute toxicity of HEOD in the treated pups during the first 24 hour period following the administration of the pesticide.

The mortality of the control pups did not change during the 24 to 48 hour period. This suggests that all the HEOD-susceptible pups were killed during the first 24 hours. In the pups treated with HEOD and glucose, on the other hand, mortality increased throughout the 24 to 48 hour period to eventually reach a level statistically equivalent to that seen in the poisoned control animals at 24 hours (Table XV).

Since no exogenous glucose was administered to the treated pups during the 24 to 48 hour period, this latter result tends to further suggest the protective effect of glucose against the acute toxicity of HEOD in the ten day old. The 1/2 life of [14 C]-glucose is 31.5 minutes in the ten day old Wistar rat (Vernon & Walker, 1972). Therefore, four hours after its administration virtually no exogenous glucose would remain in the circulation of the ten day old pup. Thus the absence of exogenous glucose during the second 24 hour period might have allowed HEOD to kill the previously protected HEOD-sensitive pups of the treated group.

E. Insulin: effects in HEOD-treated adult rats.

The increased hyperglycemia produced in the HEOD and

exogenous glucose-treated adult rat did not confer protection against HEOD. Therefore, it was decided to determine what effects hypoglycemia might have in the HEOD-exposed adult.

Adult 60 day old male rats were given a 1 LD50 dose of HEOD and two units/kg s.c. semi-lente insulin (treated group). The control group received 1 LD50 HEOD and an appropriate volume of insulin diluent. Following this, measurements of plasma glucose, hepatic glycogen and PEPCK activity, mortality and the time of onset of CNS stimulation (i.e. convulsive latency time) were made in the two groups.

Figure 5 presents the plasma glucose concentrations found in the animals during the first six hours following HEOD treatment. It is clear that insulin replaced the HEOD-induced hyperglycemia with a pronounced hypoglycemia (Table XVI). Insulin in the normal animal blocks both glycogenolysis and gluconeogenesis (Grodsky, 1977). However, hepatic PEPCK activity, two hours after HEOD was significantly increased in the treated animals of this experiment (Table XVII). Also the hepatic glycogen level was not significantly reduced in the HEOD and insulin-treated rats, although as may be seen in Table XVII, there was a trend towards lowered hepatic glycogen content.

The elevated PEPCK activity in the treated group suggests that at least at two hours after dieldrin, HEOD-induced gluconeogenesis was still operative despite the presence of insulin. That, at this time, the hypoglycemia (70 mg/dl) in the treated animals was not severe tends to support this conclusion.

Figure 5. Mean plasma glucose (mg/dl) in 60 day old male rats given 1 LD50 HEOD and either 2 U/kg s.c. semi-lente insulin (treated) or insulin diluent (control).

n: 28 rats (control)

n: 28 rats (treated)

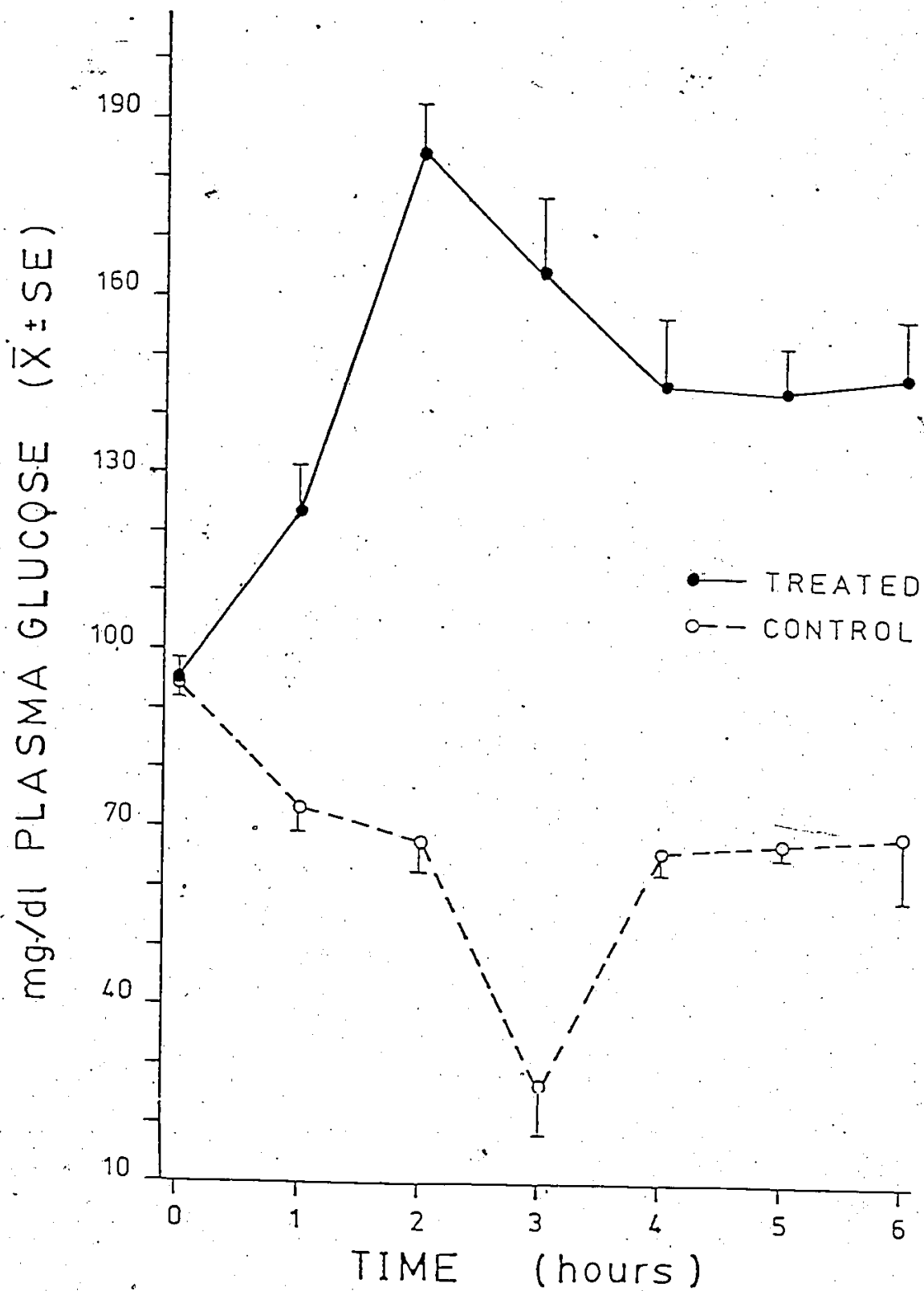


Table XVI. Plasma glucose concentrations (mg/dl) in 60 day old male rats given 1 LD50 HEOD and either, 2 U/kg s.c. semi-lente insulin (treated) or insulin diluent (control). Tabled values represent mean \pm SE.

| Time post treatment hours | CONTROL GROUP | | TREATED GROUP | | t |
|---------------------------------|---------------|------------------|---------------|-----------------|----------|
| | N rats | Mean \pm SE | N rats | Mean \pm SE | |
| 0 | 28 | 94.9 \pm 2.65 | 28 | 94.4 \pm 2.66 | 0.133 |
| 1 | 28 | 123.7 \pm 6.71 | 28 | 73.3 \pm 4.07 | 6.420 c |
| 2 | 27 | 184.4 \pm 8.47 | 25 | 67.4 \pm 4.88 | 11.951 c |
| 3 | 17 | 163.9 \pm 12.4 | 8 | 26.1 \pm 8.75 | 7.198 c |
| 4 | 14 | 145.8 \pm 9.62 | 2 | 65.7 \pm 3.35 | 3.052 b |
| 5 | 13 | 144.6 \pm 6.92 | 2 | 67.0 \pm 1.70 | 4.263 b |
| 6 | 13 | 147.7 \pm 7.77 | 2 | 69.2 \pm 12.5 | 3.778 a |

a : $P < 0.05$

b : $P < 0.01$

c : $P < 0.001$

Table XVII. Convulsion latency time (CLT), 24 hour mortality and plasma glucose, hepatic glycogen and PEPCK activity, two hours post-treatment in 60 day old male rats given 1 LD50 HEOD and either, 2 U/kg s.c. semi-lente insulin (treated) or insulin diluent (control). Tabled values represent mean \pm SE.

| A. 24 hour data. | | | | | |
|--|---------------------------|-------------------|---------------|------------------|--|
| | CONTROL GROUP | | TREATED GROUP | | |
| | N rats | Mean \pm SE | N rats | Mean \pm SE | t or χ^2 |
| CLT minutes | 19/28 ¹ | 127.9 \pm 19.12 | 21/28 | 88.1 \pm 4.85 | 2.111a |
| 24 hour mortality | 13/20 ² | 65.0% | 19/20 | 95.0% | 0.921 ³ 12.414b ⁴ |
| ----- | | | | | |
| B. 2 hour data. | | | | | |
| | (n= 8 rats/group) CONTROL | | TREATED | | |
| | | Mean \pm SE | | Mean \pm SE | t |
| Plasma glucose mg/dl | | 195.7 \pm 12.55 | | 80.0 \pm 10.91 | 6.958b |
| Hepatic glycogen ug/mg wet weight | | 13.5 \pm 5.49 | | 2.8 \pm 1.20 | 1.903 |
| Hepatic PEPCK activity Unit/g wet weight | | 1.18 \pm 0.077 | | 2.71 \pm 0.271 | 5.455b |

1 : sample size is total number of convulsive rats/group

2 : sample size excludes 8 rats/group killed at 2 hours for liver samples

3 : χ^2 value for mortality in control group versus expected mortality for 1 LD50 HEOD

4 : χ^2 value for mortality in treated group versus expected mortality for 1 LD50 HEOD

a : $P < 0.05$

b : $P < 0.001$

It should be noted however, that despite the apparent presence of HEOD-induced gluconeogenesis, insulin still prevented the development of hyperglycemia. The hormone may have achieved this by stimulating hepatic lipogenesis and/or increasing the rate of glucose uptake into extra-hepatic tissues.

Insulin also significantly increased the 24 hour mortality and significantly decreased the latency to the onset of convulsions in the treated group (Table XVII). Since mortality was increased, it is concluded that insulin induced hypoglycemia in the HEOD-exposed rat exacerbates the toxicity of the pesticide. Support for this conclusion is forthcoming from the fact that in preliminary experiments designed to determine an appropriate dose of insulin which would negate HEOD-induced hyperglycemia, insulin at a dose of two units/kg in the non-HEOD treated rat did not cause any mortality.

The above conclusion is consistent with an adaptive function for the HEOD-induced hyperglycemic response. Thus while increasing the HEOD-induced hyperglycemia with exogenous glucose conferred no adaptive advantage in the adult rat, the total negation of hyperglycemia achieved with insulin enhanced the acute toxicity of the pesticide.

Finally, the earlier onset of convulsions in the HEOD and insulin-treated animal suggests that the absence of hyperglycemia may enhance the ability of dieldrin to cause hyperstimulation in the CNS.

F. Phenobarbital: effects in HEOD-treated adult rats.

Barbiturates have been used in the ameliorative treatment of the CNS hyperstimulation resulting from organochlorine pesticide intoxication in man. We therefore determined what effect such treatment might have on the HEOD-induced carbohydrate response of the adult rat.

Adult 60 day old male rats were given a 1 LD50 dose of HEOD and 40 mg/kg i.p. phenobarbital (treated group). The control group received corn oil and an equivalent dose of phenobarbital. Measurements of plasma glucose, hepatic glycogen and PEPCK activity, mortality and time of onset of convulsions were made in the two groups.

Figure 6 presents the plasma glucose concentrations found in the control and treated groups. Only one rat of the treated group died during the 24 hour experimental period, and the plasma glucose levels of this rat are also presented.

In the control group (non-HEOD treated) the plasma glucose level declined slowly and eventually reached a stable level of 20 mg/dl by nine hours. In contrast, a significant hyperglycemia developed over this same period in the HEOD and phenobarbital-treated rats (Table XVIII).

However, the highest plasma glucose concentration found in the treated group (122 mg/dl at four hours) was only 32% greater than the original level found at zero hours. This level of hyperglycemia is considerably attenuated when compared to the level normally induced by treatment with HEOD alone. For example, as previously seen in rats given 1 LD50

Figure 6. Mean plasma glucose (mg/dl) in 60 day old male rats given 40 mg/kg i.p. sodium phenobarbital and either 1 LD50 HEOD (treated) or corn oil (control). The figure also presents the plasma glucose concentrations of the sole rat of the treated group who died.

PB. ADMIN.: phenobarbital administered
n: 14 rats (control)
n: 8 rats (treated)

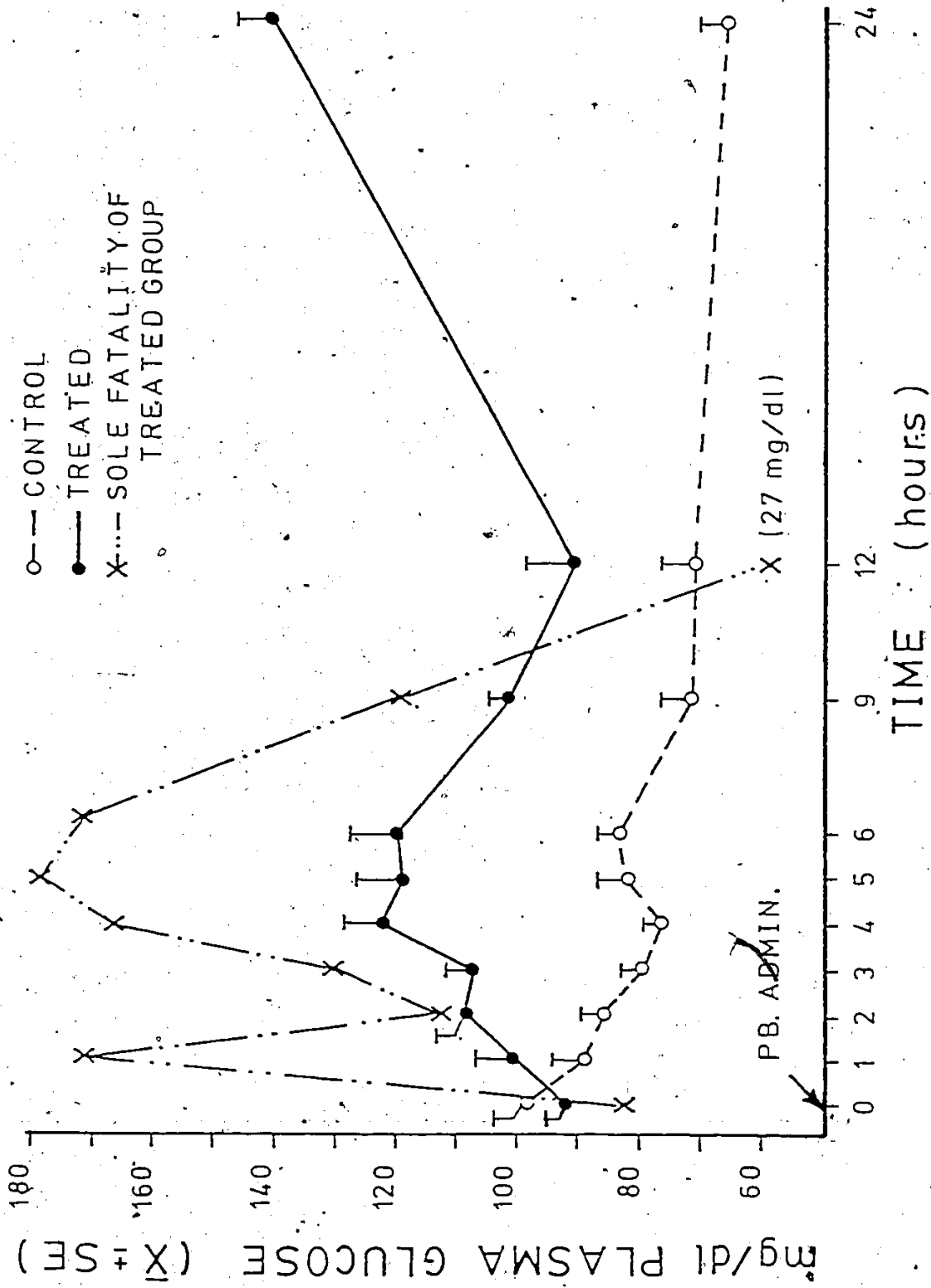


Table XVIII. Plasma glucose concentrations (mg/dl) in 60 day old male rats given phenobarbital (40 mg/kg i.p.) and either, 1 LD50 HEOD (treated) or corn oil (control). Tabled values represent mean \pm SE.

| Time post HEOD hours | CONTROL GROUP | | TREATED GROUP | | t |
|-------------------------|---------------|-----------------|---------------|------------------|--------|
| | N rats | Mean \pm SE | N rats | Mean \pm SE | |
| 0 | 10 | 99.8 \pm 3.41 | 14 | 92.4 \pm 2.14 | 1.736 |
| 1 | 10 | 89.7 \pm 3.40 | 14 | 101.3 \pm 6.21 | 1.473 |
| 2 | 10 | 86.0 \pm 3.31 | 14 | 108.4 \pm 4.99 | 3.418b |
| 3 | 6 | 79.0 \pm 3.24 | 10 | 106.8 \pm 3.78 | 5.029c |
| 4 | 6 | 76.2 \pm 3.92 | 10 | 122.0 \pm 6.38 | 5.165c |
| 5 | 6 | 82.1 \pm 2.59 | 10 | 118.5 \pm 7.57 | 3.294b |
| 6 | 6 | 83.9 \pm 4.23 | 10 | 120.4 \pm 6.56 | 3.985b |
| 9 | 6 | 72.0 \pm 2.30 | 10 | 102.2 \pm 2.83 | 7.376c |
| 12 | 6 | 71.8 \pm 4.00 | 10 | 91.0 \pm 7.45 | 1.734 |
| 24 | 6 | 67.1 \pm 3.23 | 9 | 140.4 \pm 5.52 | 9.628c |

b : $P < 0.01$

c : $P < 0.001$

of HEOD and insulin diluent, the highest plasma glucose level attained was 184 mg/dl (Figure 5). Also, in the only treatment rat of this experiment that died, the peak in plasma glucose level (i.e. 179 mg/dl at five hours) was 118% greater than the basal level at zero hours (Figure 6). Thus, phenobarbital markedly suppressed HEOD-induced hyperglycemia in the adult rat.

Phenobarbital also prevented convulsions in all the HEOD-treated rats, except for the single animal that died (Table XIX). The drug also decreased the 24 hour mortality of the treated group by 80% when compared to the 50% mortality expected on the basis of a 1 LD50 dose of HEOD. Table XIX also shows that hepatic glycogen content and PEPCK activities were unaltered in the HEOD and phenobarbital-treated rats. This latter result suggests that phenobarbital inhibited HEOD-induced gluconeogenesis.

Since phenobarbital blocked convulsive activity in the HEOD-treated rats that survived, it is concluded that the CNS depressant effect of the barbiturate predominated over the CNS stimulatory effect of dieldrin. Furthermore, since phenobarbital prevented HEOD-induced gluconeogenesis and attenuated HEOD-induced hyperglycemia, the elicitation of these responses must be controlled by the CNS.

Both the hyperglycemia and the 24 hour mortality caused by HEOD were reduced by phenobarbital. These data suggest that HEOD-induced hyperglycemia may represent a toxic, as opposed to an adaptive, response on the part of the animal to

Table XIX. Convulsive latency time (CLT), 24 hour mortality and hepatic glycogen and PEPCK activity, two hours post-treatment in 60 day old male rats given phenobarbital (40 mg/kg i.p.) and either, 1 LD50 HEOD (treated) or corn oil (control). Tabled values represent mean \pm SE.

| A. 24 hour data | | | | | | |
|--|-------------------|---------------|--|------------------|---------------|---|
| | CONTROL GROUP | | | TREATED GROUP | | |
| | N. rats | Mean \pm SE | | N. rats | Mean \pm SE | t or X^2 |
| CLT minutes | 8/10 ¹ | ∞ | | 1/14 | 720.0 | - |
| 24 hour mortality | 0/6 ² | 0.0% | | 1/10 | 10.0% | 0.000 ³ 3.910 ^a ⁴ |
| B. 2 hour data | | | | | | |
| (n= 4 rats/group) | | | | | | |
| | CONTROL | | | TREATED | | |
| | Mean \pm SE | | | Mean \pm SE | | t |
| Hepatic glycogen ug/mg wet weight | 6.0 \pm 1.04 | | | 6.8 \pm 1.48 | | 0.426 |
| Hepatic PEPCK activity Unit/g wet weight | 0.81 \pm 0.051 | | | 1.10 \pm 0.160 | | 1.726 |

1 : sample size is total number of convulsive rats/group
 2 : sample size excludes 4 rats/group killed at 2 hours for liver samples.

3 : X^2 value for mortality in control group versus expected mortality for 0 LD50 HEOD

4 : X^2 value for mortality in treated group versus expected mortality for 1 LD50 HEOD.

a : $P < 0.05$

dieldrin.

G. Exogenous d-glucose and phenobarbital: effects in HEOD-treated adult rats.

In adult rats given dieldrin and phenobarbital, the acute toxicity of HEOD was reduced perhaps as a result of a phenobarbital-induced block on the HEOD-induced carbohydrate response. If this is correct, then one should be able to increase the toxicity of the pesticide in the barbiturate-treated rat by increasing the hyperglycemia.

To test this hypothesis, 60 day old male rats were given 1 LD50 HEOD, 40 mg/kg i.p. phenobarbital and, at the times indicated in Figure 7, 6 g/kg d-glucose p.o. (treated group). Controls received corn oil, phenobarbital and exogenous glucose.

Phenobarbital did not prevent exogenous glucose from inducing hyperglycemia in the control group (Figure 7). Furthermore, the degree of hyperglycemia produced in both groups was the same during the first 12 hours of the experiment (Figure 7). Thus it is concluded that the hyperglycemia in the rat given HEOD, phenobarbital and glucose resulted from the exogenous glucose, not from the HEOD. These results support the conclusion that phenobarbital prevents HEOD-induced hyperglycemia in the adult rat.

Hepatic PEPCK activity was unaltered in the HEOD, phenobarbital and glucose-treated rats (Table XX), which indicates that HEOD-induced gluconeogenesis was blocked. Convulsive activity was seen in 33% of the treated animals and the onset

Figure 7. Mean plasma glucose (mg/dl) in 60 day old male rats given 40 mg/kg i.p. sodium phenobarbital and at the times indicated 6 g/kg p.o. d-glucose and either 1 LD50 HEOD (treated) or corn oil (control). The figure also presents, for the two groups, the percent mortality and the significance of plasma glucose differences.

ns: not significant

×: $P < 0.05$

××: $P < 0.01$

n: 6 rats (control)

n: 8 rats (treated)

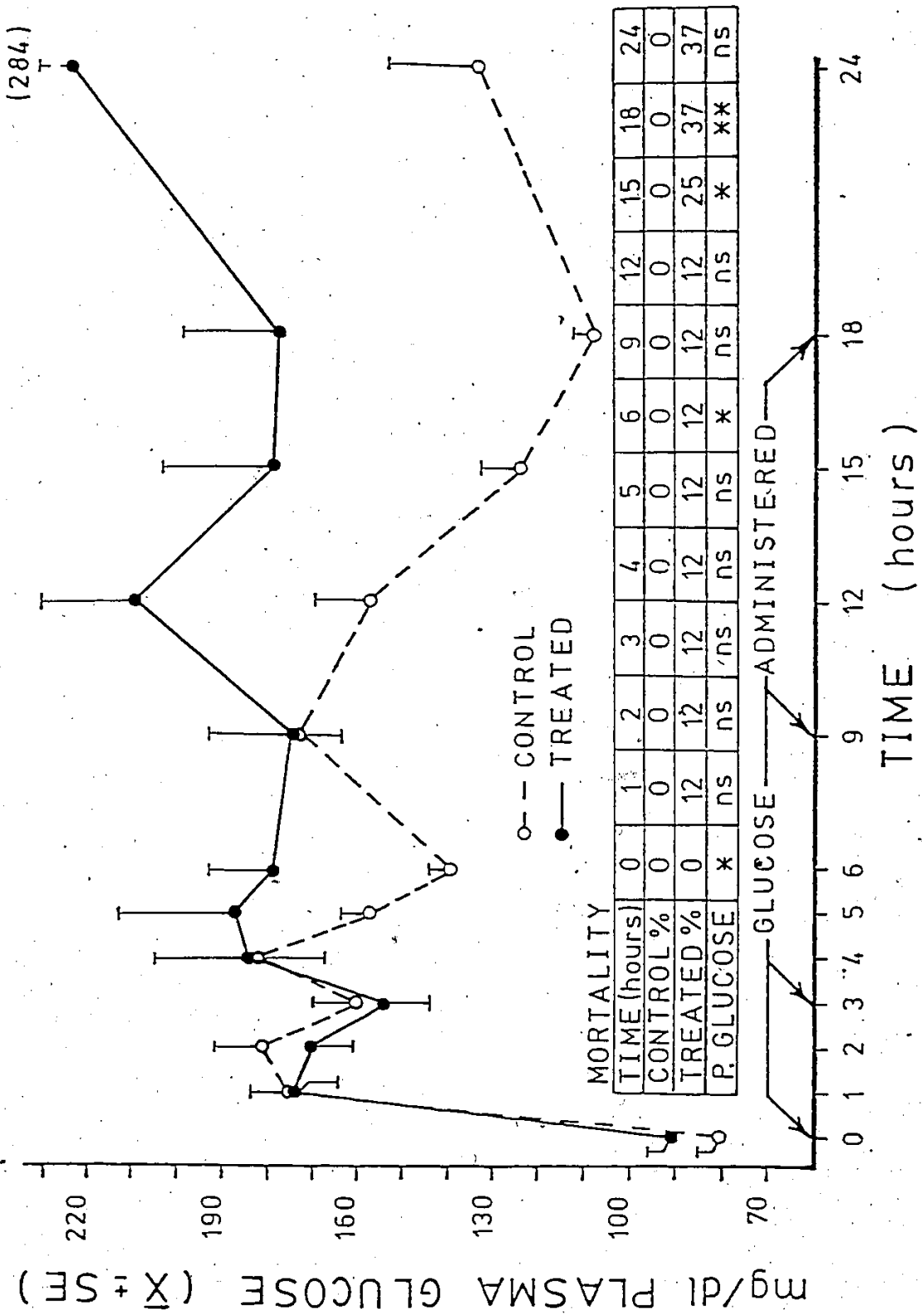


Table XX. Convulsion latency time (CLT), 24 hour mortality and hepatic glycogen and PEPCK activity, two hours post-treatment in 60 day old male rats given phenobarbital (40 mg/kg i.p.) exogenous glucose (6 g/kg p.o. at 0, 3, 9 and 18 hours after phenobarbital) and either, 1 LD50 HEOD (treated) or corn oil (control). Tabled values represent mean \pm SE.

A. 24 hour data

| | CONTROL GROUP | | TREATED GROUP | | t or X^2 |
|-------------------|-------------------|---------------|---------------|--------------------|--|
| | N rats | Mean \pm SE | N rats | Mean \pm SE | |
| CLT | 8/10 ¹ | ∞ | 4/12 | 496.8 \pm 178.57 | - |
| 24 hour mortality | 0/6 ² | 0.0% | 3/8 | 38.0% | 0.00 ³ 0.25 ⁴ |

B. 2 hour data
(n=4 rats/group)

| | CONTROL Mean \pm SE | TREATED Mean \pm SE | t |
|--------------------------------------|--------------------------|--------------------------|-------|
| Hepatic glycogen ug/mg wet weight | 15.3 \pm 3.02 | 14.8 \pm 1.66 | 0.389 |
| Hepatic PEPCK activity Unit/g | 0.671 \pm 0.147 | 0.584 \pm 0.028 | 0.580 |

1 : sample size is total number of convulsive rats/group

2 : sample size excludes 4 rats/group killed at 2 hours for liver samples

3 : X^2 value for mortality in control group versus expected mortality for 0 LD50 HEOD

4 : X^2 value for mortality in treated group versus expected mortality for 1 LD50 HEOD

of convulsions occurred at about eight hours (Table XX). In comparison, convulsions were seen in only 7% of the animals given only HEOD and phenobarbital (Table XIX) and they occurred later, (i.e. after 12 hours had elapsed). Thus the exogenous glucose stimulated HEOD-induced CNS hyperstimulation in rats in which this was depressed.

The 24 hour mortality in the HEOD, phenobarbital and glucose-treated rats was no different from that expected for a 1 LD₅₀ dose of HEOD (Table XX). Thus in these treated rats, in contrast to HEOD and phenobarbital-exposed rats, the acute toxicity of dieldrin was not attenuated. Rather, rendering HEOD and phenobarbital-treated rats hyperglycemic restored the potency of HEOD to a level equal to that seen in normal rats.

The majority (66%) of the mortality in rats given HEOD, phenobarbital and glucose occurred after 12 hours. From 9 hours onward, the plasma glucose levels in these rats increased (Figure 7). In contrast, over this same period in the controls, there was no mortality and glucose levels were decreased. These results suggest that around 9 hours, HEOD-induced gluconeogenesis was established in the treated group and that the hyperglycemia so produced contributed to the mortality.

The elimination rate constant for phenobarbital in the adult male Sprague-Dawley rat is 0.055 hr^{-1} (Butler et al., 1952). Using this rate constant, the biological half-life for the drug is 12.6 hours. Thus, late in this experiment, as the

levels of phenobarbital decreased, the CNS block on HEOD-induced gluconeogenesis was probably removed. This effect, when added to that of the exogenous glucose administered at 9 and 18 hours, produced a hyperglycemia of sufficient magnitude to restore the acute toxicity of HEOD.

H. Insulin and phenobarbital: effects in HEOD treated adult rats.

Insulin in the HEOD-exposed rat caused hypoglycemia and increased mortality. In the HEOD, phenobarbital and glucose-treated rat, the hyperglycemia induced by d-glucose increased the mortality compared to that in HEOD and phenobarbital-exposed rats. Consequently, we determined the effects of insulin on the carbohydrate response of HEOD and phenobarbital-treated rats.

Adult 60 day old male rats were administered 1 LD₅₀ HEOD, 40 mg/kg i.p. phenobarbital and 2 units/kg s.c. semi-lente insulin (treated group). Controls received corn oil, phenobarbital and insulin. Under these conditions, a pronounced hypoglycemia was found in both groups (Figure 8). Furthermore, although both the onset and the magnitude of this hypoglycemia was significantly less in the treated group during the two to four hour period, it is clear that HEOD-induced hyperglycemia was absent in the HEOD, phenobarbital and insulin-exposed animals, throughout the experiment.

Table XXI shows that hepatic glycogen levels and PEPCK activities were unchanged in the treated rat. Since hepatic PEPCK activity was unaltered, it is concluded that HEOD in

Figure 8. Mean plasma glucose (mg/dl) in 60 day old male rats given 40 mg/kg i.p. sodium phenobarbital, 2 U/kg s.c. semi-lente insulin and either 1 LD50 HEOD (treated) or corn oil (control). The figure also presents for, the two groups, the percent mortality and the significance of plasma glucose differences.

sign.: significance

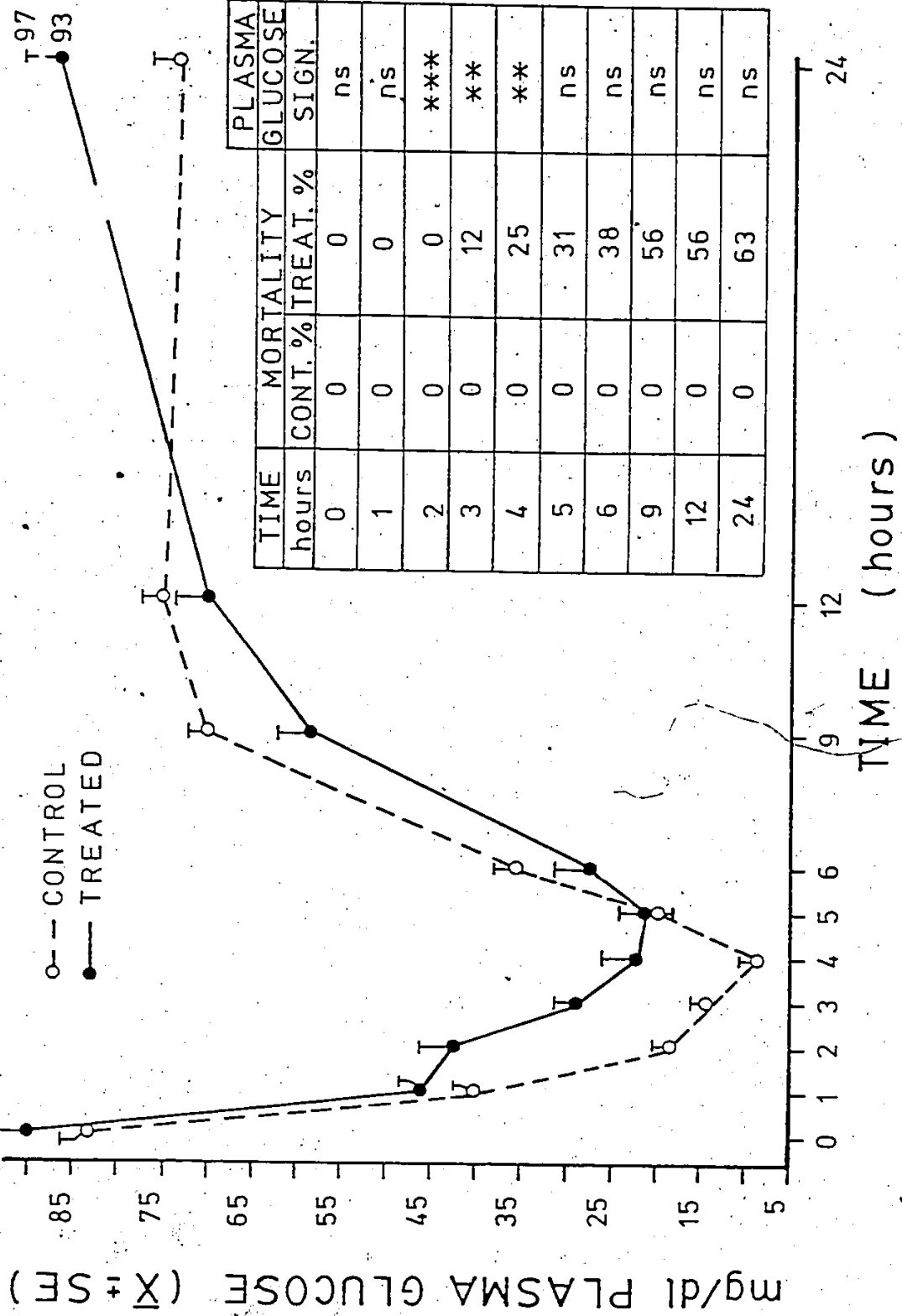
ns: not significant

xx: $P < 0.01$

xxx: $P < 0.001$

n: 16 rats (control)

n: 16 rats (treated)



| TIME hours | MORTALITY | | PLASMA GLUCOSE SIGN. |
|---------------|-----------|----------|----------------------------|
| | CONT. % | TREAT. % | |
| 0 | 0 | 0 | ns |
| 1 | 0 | 0 | ns |
| 2 | 0 | 0 | *** |
| 3 | 0 | 12 | ** |
| 4 | 0 | 25 | ** |
| 5 | 0 | 31 | ns |
| 6 | 0 | 38 | ns |
| 9 | 0 | 56 | ns |
| 12 | 0 | 56 | ns |
| 24 | 0 | 63 | ns |

Table XXI. Convulsion latency time (CLT), 24 hour mortality and hepatic glycogen and PEPCK activity, two hours post-treatment in 60 day old male rats given phenobarbital (40 mg/kg i.p.) semi-lente insulin (2 U/kg s.c.) and either, 1 LD50 HEOD (treated) or corn oil (control). Tabled values represent mean \pm SE.

| A. 24 hour data | | | | | |
|--|-------------------|---------------|------------------|-------------------|---|
| | CONTROL GROUP | | TREATED GROUP | | t or χ^2 |
| | N rats | Mean \pm SE | N rats | Mean \pm SE | |
| CLT | 8/20 ¹ | ∞ | 13/20 | 154.2 \pm 14.31 | -- |
| 24 hour mortality | 0/16 ² | 0.0% | 10/16 | 62.5% | 0.00, ³ 0.51 ⁴ |
| ----- | | | | | |
| B. 2 hour data (n=4 rats/group) | | | | | |
| | CONTROL | | TREATED | | t |
| | Mean \pm SE | | Mean \pm SE | | |
| Hepatic glycogen ug/mg wet weight | 0.16 \pm 0.01 | | 0.17 \pm 0.02 | | 0.882 |
| Hepatic PEPCK activity Unit/g wet weight | 1.75 \pm 0.116 | | 2.25 \pm 0.412 | | 1.168 |
| ----- | | | | | |

1 : sample size is total number of convulsive rats/group
 2 : sample size excludes 4 rats/group killed at 2 hours for liver samples

3 : X^2 value for mortality in control group versus expected mortality for 0 LD50 HEOD

4 : X^2 value for mortality in treated group versus expected mortality for 1 LD50 HEOD

these animals did not stimulate a gluconeogenic response. Since HEOD induced a gluconeogenic response in the HEOD and insulin-treated rat, as evidenced by increased hepatic PEPCK activity (Table XVII), it is thought that in the HEOD, phenobarbital and insulin-treated animal, the gluconeogenic inhibition was caused by phenobarbital. A phenobarbital-induced inhibition of HEOD-elicited gluconeogenesis was also inferred for rats given either, HEOD and phenobarbital (Table XIX) or HEOD, phenobarbital and glucose (Table XX).

As a consequence of this inhibition of HEOD-induced gluconeogenesis caused by phenobarbital, one would expect hypoglycemia in the HEOD, phenobarbital and insulin-treated rat to be increased when compared to that found in HEOD and insulin-treated animals. The data in Table XXII shows that the degree of hypoglycemia caused by HEOD, phenobarbital and insulin-treatment was indeed greater than that produced by HEOD and insulin-treatment.

Insulin, in the HEOD-treated rat, caused hypoglycemia and increased mortality (Table XVII). On the other hand, phenobarbital in the HEOD-exposed rat attenuated the hyperglycemic response and decreased the mortality (Table XIX). Since phenobarbital increased the insulin-induced hypoglycemia in HEOD and insulin-treated rats, one might also expect the mortality to be increased in these animals, when compared to that in rats given only HEOD and insulin. However, as Table XXIII shows, this was not the case. When the mortality in the HEOD, phenobarbital and insulin-treated rats was compared to that of rats

Table XXII. Results of 2-way anova on mean plasma glucose concentrations (mg/dl) during first six hours of experimentation in 60 day old male rats treated with either, 1 LD50 HEOD and 2 U/kg s.c. semi-lente insulin (data of treated group Table XVI) or HEOD, insulin and 40 mg/kg i.p. phenobarbital. Tabled values represent mean \pm SE.

| Time post treatment hours | TREATMENT GROUP | | | |
|---------------------------------|-----------------------------|-----------------|---------------------|-----------------|
| | HEOD + Insulin ¹ | | HEOD + Insulin + PB | |
| | N rats | Mean \pm SE | N rats | Mean \pm SE |
| 0 | 28 | 94.4 \pm 2.66 | 20 | 90.1 \pm 1.70 |
| 1 | 28 | 73.3 \pm 4.07 | 20 | 45.9 \pm 2.80 |
| 2 | 25 | 67.3 \pm 4.99 | 19 | 43.5 \pm 3.13 |
| 3 | 8 | 26.1 \pm 8.75 | 14 | 28.8 \pm 5.02 |
| 4 | 2 | 65.7 \pm 3.35 | 12 | 23.3 \pm 5.19 |
| 5 | 2 | 67.0 \pm 1.70 | 11 | 20.3 \pm 6.54 |
| 6 | 2 | 69.2 \pm 12.5 | 10 | 27.4 \pm 8.42 |

| 2-way anova | | df | Mean square | F |
|--------------|----|---------|-------------|---|
| Source | | | | |
| ----- | | | | |
| Treatments | 1 | 2413.03 | 12.880 a | |
| Time (hours) | 6 | 808.23 | 4.315 a | |
| Error | 6 | 187.30 | | |
| Total | 13 | | | |

PB : phenobarbital

1 : data of treated group Table XVI

a : $P < 0.05$

Table XXIII. Data comparison - Convulsion latency time (CLT), 24 hour mortality and hepatic glycogen and PEPCK activity, two hours after treatment with either, 1 LD50 HEOD and 2 U/kg s.c. semi-lente insulin (data of treated group Table XVII) or HEOD, insulin and 40 mg/kg i.p. phenobarbital (data of treated group Table XXI). Tabled values represent mean \pm SE.

| A. 24 hour data | | | | | |
|---|-----------------------------|------------------|----------------------------------|------------------|----------------------|
| | HEOD + Insulin ¹ | | HEOD + Insulin + PB ² | | |
| | N rats | Mean \pm SE | N rats | Mean \pm SE | t or χ^2 |
| CLT | 21/28 ³ | 88.1 \pm 4.85 | 13/20 | 154.2 \pm 14.3 | 4.375 b |
| 24 hour mortality | 19/20 ⁴ | 95.0% | 10/16 | 62.5% | 5.998 a ⁵ |
| B. 2 hour data | | | | | |
| | HEOD + Insulin | | HEOD + Insulin + PB | | |
| | N rats | Mean \pm SE | N rats | Mean \pm SE | t |
| Hepatic glycogen ug/mg wet weight | 8 | 2.8 \pm 1.20 | 4 | 0.17 \pm 0.02 | 2.198 |
| Hepatic PEPCK activity Units/g wet weight | 8 | 2.71 \pm 0.271 | 4 | 2.25 \pm 0.412 | 0.956 |

PB : phenobarbital

1 : data of treated group Table XVII

2 : data of treated group Table XXI

3 : sample size is total number of convulsive rats/group

4 : sample size excludes 8 or 4 rats/group killed at two hours for liver samples

5 : χ^2 value for mortality in HEOD + Insulin + PB group versus mortality in HEOD + Insulin group

a : $P < 0.05$

b : $P < 0.01$

given only HEOD and insulin, significantly less mortality was found in the former group. Furthermore, the mortality suffered by the HEOD, phenobarbital and insulin-treated animals was no different from that which would have been expected if these rats had received only a 1 LD50 dose of dieldrin (Table XXI). Therefore, it is concluded that in the HEOD and insulin-exposed rat, phenobarbital attenuates the enhanced HEOD toxicity caused by insulin. These results further suggest that phenobarbital produces this effect by a mechanism other than causing a decrease in HEOD-induced gluconeogenesis.

Insulin, in the HEOD-treated rat facilitates HEOD-induced convulsions, as evidenced by an earlier onset of convulsive activity (Table XVII). Phenobarbital will delay HEOD-induced convulsive activity in rats given either, dieldrin (Table XIX) or HEOD and glucose (Table XX). Therefore, insulin enhances the ability of HEOD to cause CNS hyperstimulation, while phenobarbital inhibits this ability. Table XXIII shows that the onset of convulsions in the HEOD, phenobarbital and insulin-treated rat was delayed, by 75%, compared to that found in the HEOD and insulin treated animal. Thus, phenobarbital inhibits the ability of insulin to enhance HEOD-induced CNS hyperstimulation in the rat. Therefore, it is thought that it is the ability of the drug to decrease HEOD-induced CNS hyperstimulation, and not the ability to attenuate the HEOD-induced hyperglycemic response, which ultimately served to protect the HEOD, phenobarbital and insulin-exposed rats from insulin-enhanced HEOD toxicity.

1. 2-Deoxyglucose: effects in HEOD treated adult rats.

According to Houpt & Hance (1977) treatment of the normal animal with 2-deoxyglucose, a glucose analogue which is not metabolized, causes two primary effects. First, as a result of the competition between 2-deoxyglucose and glucose for glycolytic enzymes, a functional intracellular glucose deficiency develops in the body tissues. Second, primarily as a consequence of glucose deficiency in the CNS, sympathetic nervous activity and adrenal catecholamine release are both stimulated. As a result, gluconeogenesis and glycogenolysis are enhanced and hyperglycemia develops.

If hyperglycemia had adaptive value in the HEOD-treated rat, then one might expect increased mortality to result as a consequence of the intracellular glucose deficiency induced by 2-deoxyglucose, in the HEOD and 2-deoxyglucose-treated rat. Conversely, if HEOD-induced hyperglycemia produced a toxic effect, by elevating intracellular glucose levels in the HEOD-treated rat, then 2-deoxyglucose-induced intracellular glucose deficiency should reduce the mortality suffered by these animals.

Consequently, the effect of 2-deoxyglucose on the hyperglycemia and mortality of the HEOD-treated rat was assessed. Adult male rats were given 1 LD₅₀ HEOD and either 1 g/Kg s.c. 2-deoxyglucose (treated group) or distilled water (control group).

The plasma glucose level in the control group rose steadily to reach a peak level of 159.3 mg/dl, two hours after HEOD

administration (Figure 9). The plasma glucose level then declined over the next four hours to reach 112.4 mg/dl. From six to 24 hours, the plasma glucose level remained stable.

The plasma glucose level in HEOD and 2-deoxyglucose-treated animals also rose dramatically during the first two hours following HEOD, to reach a peak level of 321.1 mg/dl. During the next seven hours, the plasma glucose level fell steadily in the treated group to ultimately reach a level of 93.5 mg/dl. For the remainder of the experiment the plasma glucose level remained stable.

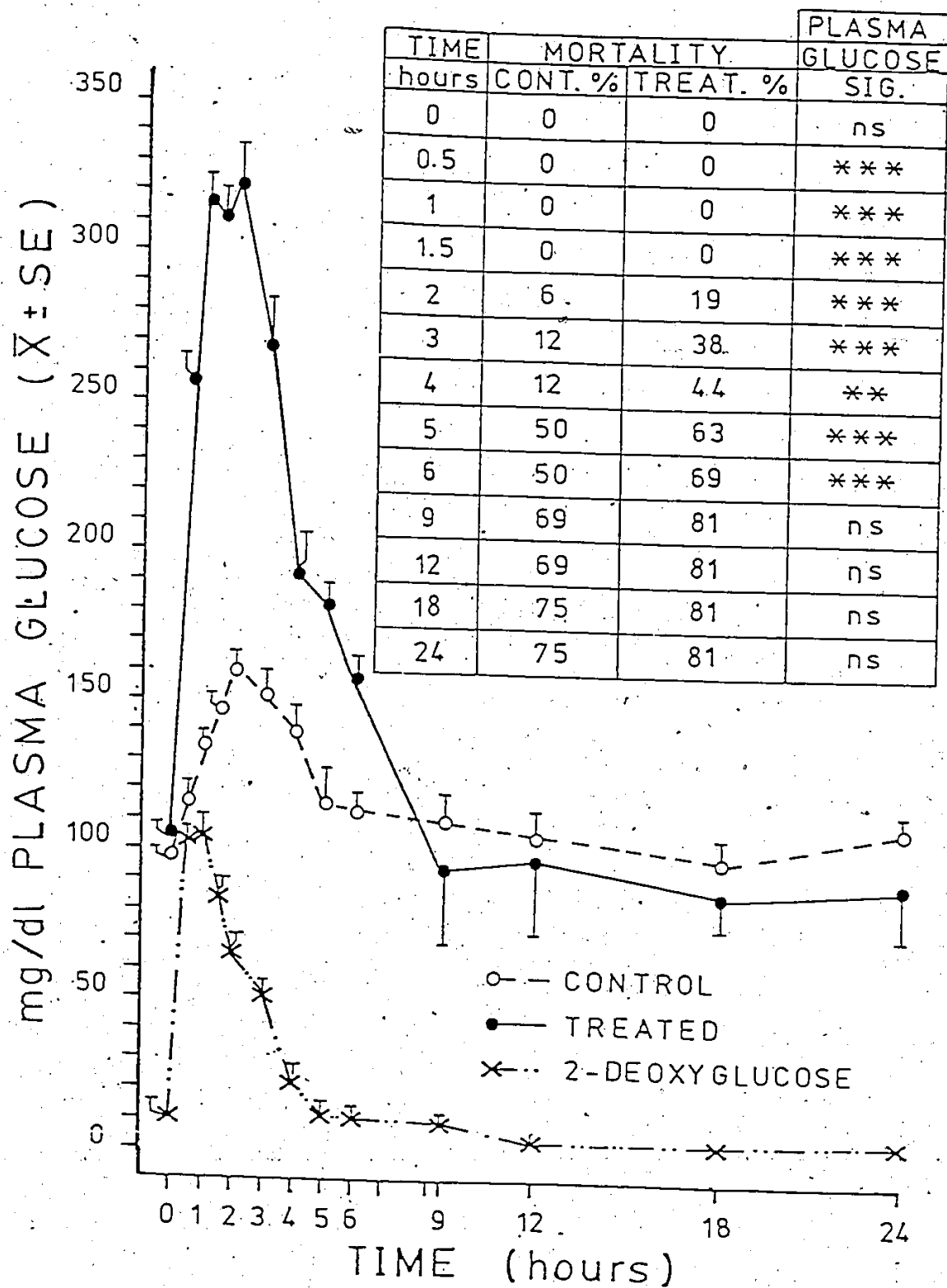
Figure 9 also presents the plasma 2-deoxyglucose concentrations of the HEOD and 2-deoxyglucose-treated rats. In these animals, the plasma 2-deoxyglucose level increased immediately following experimental treatment, to reach a peak level of 106.7 mg/dl at one hour. During the next five hours, the plasma 2-deoxyglucose level declined rapidly and eventually reached a plasma concentration of only 11.0 mg/dl, by six hours. Thereafter, 2-deoxyglucose was virtually undetectable for the remainder of the experiment.

Plasma glucose levels between the two experimental groups differed significantly from one-half to six hours. The plasma glucose level in the HEOD and 2-deoxyglucose-treated rat was always greater (by 98%) than that in the control animal. Thus, as expected, 2-deoxyglucose potentiated the hyperglycemia induced by HEOD in the treated group.

However, it is important to note that the glucose oxidase assay for plasma glucose is subject to false positive effects

Figure 9. Mean plasma glucose (mg/dl) in 60 day old male rats given 1 LD50 HEOD and either 1 g/kg s.c. 2-deoxyglucose (treated) or distilled water (control). The figure also presents mean plasma 2-deoxyglucose (mg/dl) in the treated group and for the two groups, the percent mortality and the significance of plasma glucose differences.

sig.: significance
ns: not significant
xx: $P < 0.01$
xxx: $P < 0.001$



when 2-deoxyglucose is present in the sample (Kingsley & Getchell, 1960). The extent of 2-deoxyglucose interference was not determined here, but is likely to be of little importance. For example, Houpt & Hance (1977) determined that the apparent glucose concentration in dog serum, as assayed by the glucose oxidase method, is increased by only 14 mg/dl for every 100 mg/dl of 2-deoxyglucose present. This is equivalent to only a 14% increase in the assayed plasma glucose level, if the normal fasting plasma glucose level in the dog approximates 100 mg/dl, and 100 mg/dl of 2-deoxyglucose was also present.

However, even if 100% of the 2-deoxyglucose present in the plasma is measured as glucose, the interference produced by the 2-deoxyglucose does not change the significance of our results. Thus, with 100% interference, one may subtract the plasma levels of 2-deoxyglucose from the corresponding plasma "glucose" levels, to obtain the corrected or "true" plasma glucose levels (Table XXIV). As is shown in Table XXIV, the corrected plasma glucose levels in the treated rats are still always significantly greater than the levels in the control rats. Thus 2-deoxyglucose significantly increased the HEOD-induced hyperglycemia and by inference would also have produced an intracellular glucose deficiency.

On the other hand, this treatment did not alter the time at which convulsive activity first occurred, the number of rats which exhibited convulsions or the 24 hour mortality in the HEOD-exposed rat (Table XXV). Also, the drug had no effect on the pattern of mortality induced by dieldrin (Table XXVI).

Table XXIV. Derivation of corrected mean plasma glucose (i.e. "glucose - 2-deoxyglucose") in 60 day old male rats given 1 LD50 HEOD and either, 1 g/kg s.c. 2-deoxyglucose (treated) or distilled water (control) and results of 2-way paired anova on corrected plasma glucose of treated rats versus plasma glucose of control rats.

A. Derivation of corrected mean plasma glucose for treated rats.

| Time post HEOD hours | Treated Mean plasma gluc. (mg/dl) | Treated Mean 2-deoxygluc. (mg/dl) | Corrected Mean plasma gluc. (mg/dl) | Control Mean plasma gluc. (mg/dl) |
|----------------------|-----------------------------------|-----------------------------------|-------------------------------------|-----------------------------------|
| 0.5 | 256.7 | 105.4 | 151.3 | 104.2 |
| 1.0 | 316.2 | 106.7 | 209.5 | 134.0 |
| 1.5 | 311.2 | 88.0 | 223.2 | 146.2 |
| 2.0 | 321.1 | 67.6 | 253.5 | 159.3 |
| 3.0 | 268.2 | 51.5 | 216.7 | 150.6 |
| 4.0 | 191.6 | 23.2 | 168.4 | 138.5 |
| 5.0 | 182.2 | 11.6 | 170.6 | 115.5 |
| 6.0 | 156.7 | 10.2 | 146.5 | 112.4 |

B. Results of 2-way paired anova (corrected versus control)

| Source | df | Mean square | F |
|-----------------------------|----|-------------|---------|
| Treatments (plasma glucose) | 1 | 14307.6 | 57.32 c |
| Time (hours) | 7 | 1632.9 | 6.54 a |
| Error | 7 | | |
| Total | 15 | | |

gluc. : glucose
a : $P < 0.05$

2-deoxygluc. : 2-deoxyglucose
c : $P < 0.001$

Table XXV. Convulsion latency time (CLT) and 24 hour mortality in 60 day old male rats given 1 LD50 HEOD and either, 1 g/kg s.c. 2-deoxyglucose (treated) or distilled water (control). Tabled values represent mean \pm SE.

| | CONTROL GROUP | | TREATED GROUP | | t or X^2 |
|----------------------|--------------------|------------------|---------------|------------------|--|
| | N rats | Mean \pm SE | N rats | Mean \pm SE | |
| CLT minutes | 16/16 ¹ | 189.6 \pm 18.0 | 15/16 | 136.7 \pm 23.4 | 1.804 |
| 24 hour mortality | 12/16 | 75.0% | 13/16 | 81.3% | 1.200 ² 2.327 ³ |

1 : sample size is total number of convulsive rats/group

2 : X^2 value for mortality in control group versus expected mortality for 1 LD50 HEOD

3 : X^2 value for mortality in treated group versus expected mortality for 1 LD50 HEOD

Table XXVI. Mortality in 60 day old male rats given 1 LD50 HEOD and either, 1 g/kg s.c. 2-deoxyglucose (treated) or distilled water (control).

| Time post HEOD hours | CONTROL MORTALITY | | TREATED MORTALITY | | χ^2 * |
|----------------------------|-------------------|------------|-------------------|------------|------------|
| | N rats | as percent | N rats | as percent | |
| 0.0 | 0/16 | 0.0 | 0/16 | 0.0 | - |
| 0.5 | 0/16 | 0.0 | 0/16 | 0.0 | - |
| 1.0 | 0/16 | 0.0 | 0/16 | 0.0 | - |
| 1.5 | 0/16 | 0.0 | 0/16 | 0.0 | - |
| 2.0 | 1/16 | 6.3 | 3/16 | 18.8 | 0.286 |
| 3.0 | 2/16 | 12.5 | 6/16 | 37.5 | 1.500 |
| 4.0 | 2/16 | 12.5 | 7/16 | 43.8 | 2.473 |
| 5.0 | 8/16 | 50.0 | 10/16 | 62.5 | 0.127 |
| 6.0 | 8/16 | 50.0 | 11/16 | 68.8 | 0.518 |
| 9.0 | 11/16 | 68.8 | 13/16 | 81.3 | 0.167 |
| 12.0 | 11/16 | 68.8 | 13/16 | 81.3 | 0.167 |
| 18.0 | 12/16 | 75.0 | 13/16 | 81.3 | 0.183 |
| 24.0 | 12/16 | 75.0 | 13/16 | 81.3 | 0.183 |

* : χ^2 values were corrected for continuity - Yates correction

Therefore, the inferred intracellular glucose deficiency produced in the CNS by 2-deoxyglucose did not change the acute toxicity of HEOD.

This situation is somewhat analogous to that seen in the HEOD and d-glucose treated rat. Recall that in animals so treated, although the subsequent hyperglycemia was markedly elevated, no significant change in the 24 hour mortality caused by dieldrin was detected. Potentiating the hyperglycemia in these animals may also have increased the intracellular CNS glucose concentration.

Thus increasing the hyperglycemia with d-glucose, or decreasing the CNS glucose concentration with 2-deoxyglucose in HEOD-exposed rats confers no adaptive advantage to the animal.

J. Atropine: effects in HEOD treated adult rats.

Atropine by blocking the effects of vagal stimulation of the heart has been shown to reduce the toxicity of dieldrin (Gowdy et al., 1954). Since the HEOD-induced carbohydrate response is associated with the toxicity of HEOD, it was decided to determine what effect atropine might have on HEOD-induced hyperglycemia and mortality.

Figure 10 presents the plasma glucose levels found in adult rats given 1 LD50 HEOD and either 4 mg/kg i.p. atropine (treated) or 0.9% saline (control). Atropine significantly reduced the HEOD-induced hyperglycemia during the first four hours of the experiment. After that time, atropine did not continue to attenuate the hyperglycemia, despite additional

Figure 10. Mean plasma glucose (mg/dl) in 60 day old male rats given 1 LD50 HEOD and either 4 mg/kg s.c. atropine (treated) or 0.9% saline (control). The figure also presents, for the two groups, the percent mortality and the significance of plasma glucose differences.

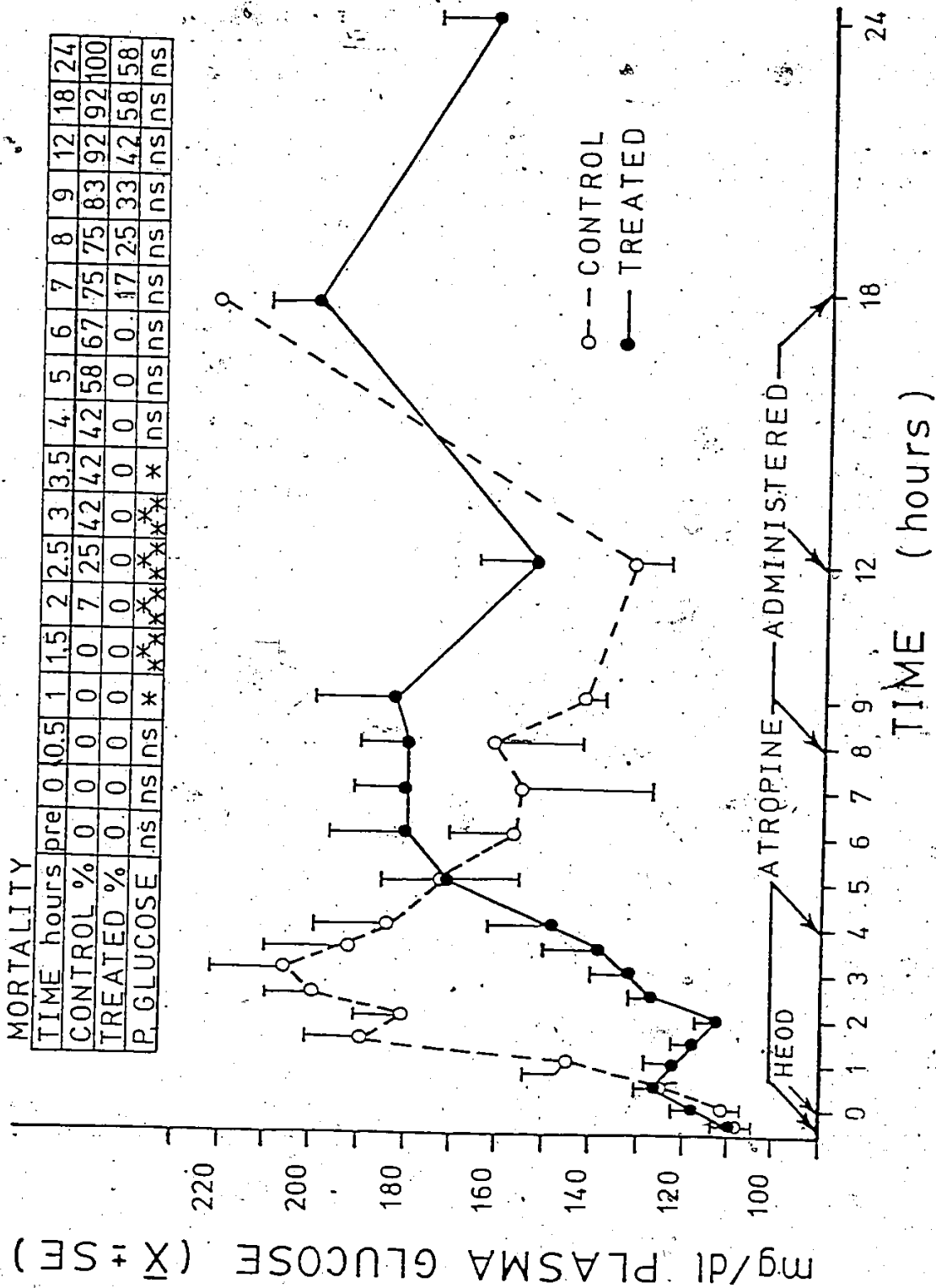
ns: not significant

x: $P < 0.05$

xxx: $P < 0.001$

n: 12 rats (control)

n: 12 rats (treated)



doses of the drug at 4, 8, 12 and 18 hours. Thus from four hours onward, the plasma glucose levels in the two groups were no different. Figure 10 also shows that mortality in the treated group did not begin to occur until seven hours had elapsed. In contrast, rats given HEOD and saline began to die after only two hours of the experiment had passed.

Atropine significantly increased (by 120%) the latency to convulsions and decreased (by 64%) the number of rats exhibiting convulsion (Table XXVII). The drug did not affect either the hepatic glycogen content or PEPCK activity.

Figure 10 shows that the pattern of mortality seen in the two groups was different. Therefore, the mortality patterns of the two groups were statistically compared through a series of χ^2 tests (Table XXVIII). It was found that at three hours, the mortality rates of the two groups diverged with a significantly higher rate occurring in the HEOD and saline-treated group. From three hours until termination of the experiment at 24 hours, the mortality in the HEOD and atropine-treated rats was always significantly less than that in the controls.

Thus in toto, significantly fewer HEOD and atropine-treated rats died than did HEOD and saline-treated rats. However, the drug did not alter the toxicity of HEOD when compared to that expected for a 1 LD₅₀ dose of the pesticide (Table XXVII). There is no reason to believe that, in the absence of atropine the rats of the HEOD and atropine group would not have had a mortality level equivalent to that of the control animals. Therefore, it is concluded that atropine

Table XXVII. Convulsion latency time (CLT), 24 hour mortality and hepatic glycogen and PEPCK activity, two hours post-treatment in 60 day old male rats given 1 LD50 HEOD and either, 4 mg/kg s.c. atropine (treated) or 0.9% saline (control) at: 40 minutes prior to, and 4, 8, 12 and 18 hours after HEOD. Tabled values represent mean \pm SE.

| A. 24 hour data | | | | | |
|--|--------------------------|------------------|--------------------------|------------------|--|
| CONTROL GROUP | | | TREATED GROUP | | |
| | N rats | Mean \pm SE | N rats | Mean \pm SE | t or X^2 |
| CLT minutes | 11/16 ¹ | 122.0 \pm 22.5 | 4/16 | 272.0 \pm 14.7 | 5.576 b |
| 24 hour mortality | 12/12 ² | 100.0% | 7/12 | 58.3% | 8.000 b ³ 0.170 ⁴ |
| B. 2 hour data (n=4 rats/group) | | | | | |
| | CONTROL Mean \pm SE | | TREATED Mean \pm SE | | t |
| Hepatic glycogen ug/mg wet weight | 34.7 \pm 10.1 | | 23.8 \pm 7.18 | | 0.622 |
| Hepatic PEPCK activity Unit/g wet weight | 1.23 \pm 0.151 | | 1.26 \pm 0.085 | | 1.028 |

1 : sample size is total number of convulsive rats/group

2 : sample size excludes 4 rats/group killed at 2 hours for liver samples

3 : X^2 value for mortality in control group versus expected mortality for 1 LD50

4 : X^2 value for mortality in treated group versus expected mortality for 1 LD50

b : $P < 0.01$

Table XXVIII. Mortality in 60 day old male rats given 1 LD50 HEOD and either, 4 mg/kg s.c. atropine (treated) or 0.9% saline (control) at: 40 minutes prior to, and 4, 8, 12 and 18 hours after HEOD.

| Time post HEOD hours | CONTROL MORTALITY | | TREATED MORTALITY | | χ^2 * |
|----------------------------|-------------------|------------|-------------------|------------|------------|
| | N rats | as percent | N rats | as percent | |
| 0.0 | 0/12 | 0.0 | 0/12 | 0.0 | - |
| 0.5 | 0/12 | 0.0 | 0/12 | 0.0 | - |
| 1.0 | 0/12 | 0.0 | 0/12 | 0.0 | - |
| 1.5 | 0/12 | 0.0 | 0/12 | 0.0 | - |
| 2.0 | 2/12 | 17.0 | 0/12 | 0.0 | 0.015 |
| 2.5 | 3/12 | 25.0 | 0/12 | 0.0 | 0.595 |
| 3.0 | 5/12 | 42.0 | 0/12 | 0.0 | 4.042 a |
| 3.5 | 5/12 | 42.0 | 0/12 | 0.0 | 4.042 a |
| 4.0 | 5/12 | 42.0 | 0/12 | 0.0 | 4.042 a |
| 5.0 | 7/12 | 58.0 | 0/12 | 0.0 | 7.261 b |
| 6.0 | 8/12 | 67.0 | 0/12 | 0.0 | 9.188 b |
| 7.0 | 9/12 | 75.0 | 2/12 | 17.0 | 6.042 a |
| 8.0 | 9/12 | 75.0 | 3/12 | 25.0 | 4.167 a |
| 9.0 | 10/12 | 83.0 | 4/12 | 33.0 | 4.287 a |
| 12.0 | 11/12 | 92.0 | 5/12 | 42.0 | 4.688 a |
| 18.0 | 11/12 | 92.0 | 7/12 | 58.0 | 2.000 |
| 24.0 | 12/12 | 100.0 | 7/12 | 58.0 | 4.042 a |

* : χ^2 values were corrected for continuity - Yates correction

a : $P < 0.05$

b : $P < 0.01$

did significantly attenuate the toxicity of the pesticide.

A 1 LD50 dose of HEOD usually causes a peak hyperglycemic response, of 170-190 mg/dl, two to three hours after administration to the 60 day old rat. For example, the peak plasma glucose level seen in rats given HEOD and insulin diluent was 185 mg/dl, two hours post-treatment (Figure 5). Similarly, the peak plasma glucose level found at three hours in the HEOD and saline-treated rats of this experiment was 206 mg/dl (Figure 10).

Furthermore, HEOD-induced hyperglycemia does not usually remain at peak levels for more than one hour. However, in the control animals of this experiment, plasma glucose remained in excess of 180 mg/dl for a period of three hours (Figure 10). Thus there exists a good correlation between prolonged HEOD-induced hyperglycemia and the greatly increased mortality seen in these rats (i.e. twice that expected for 1 LD50 HEOD).

It is also interesting that the onset of mortality in the HEOD and atropine group also correlates well with the establishment of "significant" hyperglycemia (i.e. a plasma glucose concentration in excess of 180 mg/dl). This result suggests that atropine may have attenuated the toxicity of HEOD by delaying the onset of HEOD-induced hyperglycemia.

The onset of convulsive activity and presumably therefore, the initiation of significant vagal hyperstimulation occurred at four and one-half hours in the HEOD and atropine-treated rats. However, despite this enhanced vagal activity, mortality in these animals did not occur until seven hours had passed, a

time at which the plasma glucose level was equal to 181 mg/dl. This result supports the conclusion presented above.

The mechanism by which atropine prevented HEOD-induced hyperglycemia is unknown. However, electrical stimulation of the vagus will cause increased pancreatic release of glucagon and this effect can be blocked by atropine (Gerich et al., 1976). Since atropine inhibited convulsive activity during the first four and one-half hours of the experiment, vagal hyperactivity was probably inhibited during that time. Therefore atropine may have prevented the immediate HEOD-induced hyperglycemic response by inhibiting glucagon release.

K. L-a-methyldopa: effects in HEOD treated adult rats.

Atropine inhibited the initial induction of hyperglycemia and decreased mortality in the dieldrin-exposed rat. These results indicate that parasympathetic activity is required for both the carbohydrate response and the toxicity caused by HEOD. It was decided therefore to determine if central sympathetic activity also played a role in the etiology of these effects. L-a-methyldopa, which depletes norepinephrine in sympathetic axons, was chosen to effect a decrease in central sympathetic outflow in the HEOD-treated rat.

Adult male rats were given 1 LD50 HEOD and either 200 mg/kg s.c. L-a-methyldopa (treated) or acid-saline (control) at the times indicated in Figure 11. L-a-methyldopa caused an immediate 25% decrease in the plasma glucose concentration of the treated group during the first 30 minutes of the experiment (Figure 11). Thereafter, plasma glucose levels in the treated

Figure 11. Mean plasma glucose (mg/dl) in 60 day old male rats given 1 LD50 HEOD and either 200 mg/kg i.p. L-a-methyldopa (treated) or acid-saline (control). The figure also presents, for the two groups, the percent mortality and the significance of plasma glucose differences.

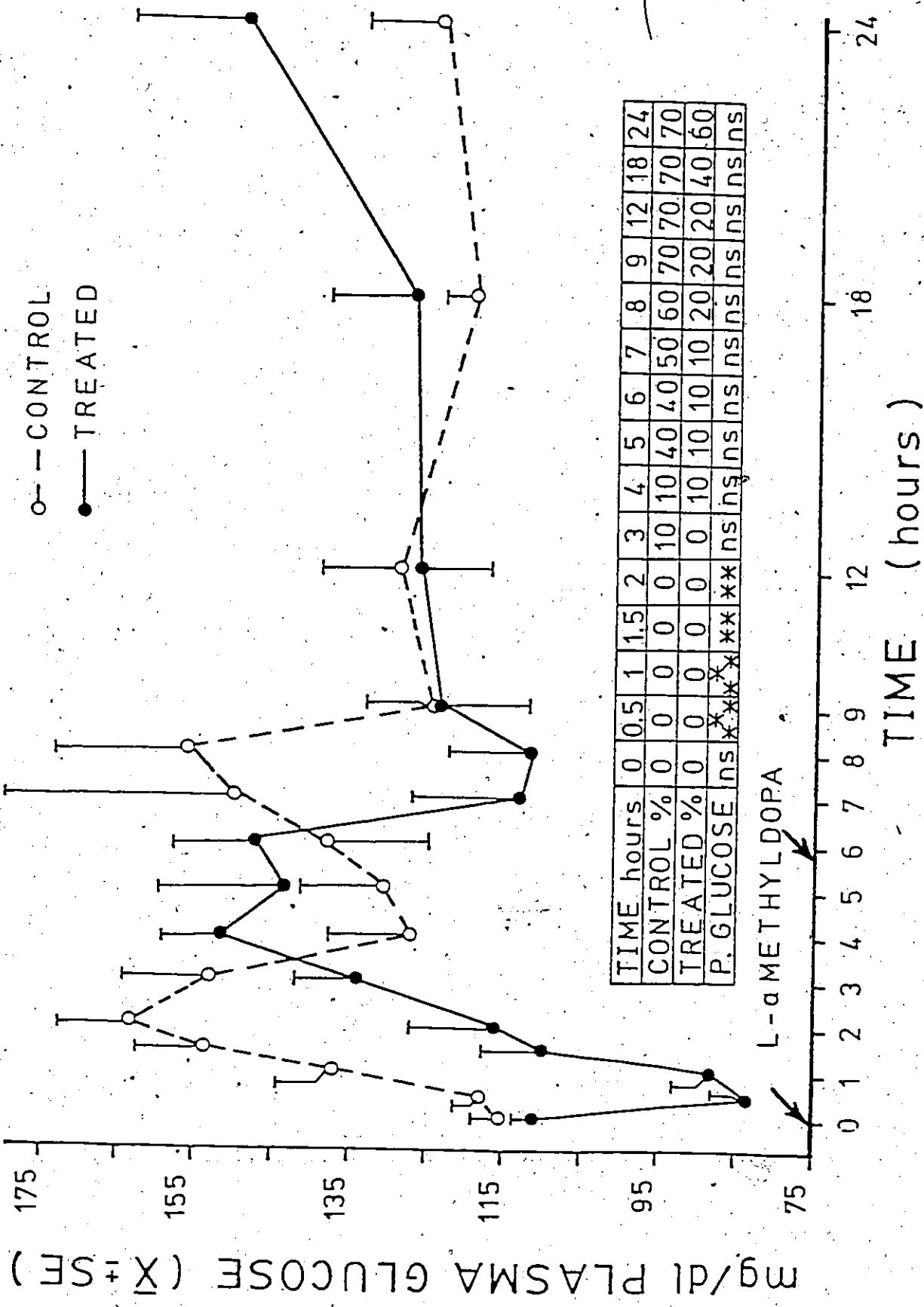
ns: not significant

xx: $P < 0.01$

xxx: $P < 0.001$

n: 10 rats (control)

n: 10 rats (treated)



group rose steadily. However they did not reach levels equivalent to those seen in the control group until two and one-half hours of the experiment had passed. Furthermore, the peak plasma glucose level in the treated group (153 mg/dl) was not attained until four hours. In contrast, in the controls the peak plasma glucose level (167 mg/dl) occurred at two hours. Thus L-a-methyldopa effectively delayed the HEOD-induced hyperglycemic response by about two hours.

Between four and six hours, both groups maintained relatively stable and equivalent plasma glucose concentrations. At six hours, the treated group received a second dose of L-a-methyldopa. The controls were given acid saline at this time. This L-a-methyldopa dose also depressed the plasma glucose level and did so for about two hours. In contrast, the plasma glucose level in the controls increased during this time. Beginning at eight hours, the plasma glucose level of the treated group rose and that of the control group fell, to reach an equivalent level by nine hours. From nine hours onward, the plasma glucose levels of the two groups remained equivalent.

In spite of the apparent differences in plasma glucose just described, statistically significant differences between the two groups of rats were demonstrable only during the first two hours of the experiment. L-a-methyldopa did not alter the time at which HEOD-induced convulsions occurred (Table XXIX). However, the drug reduced the total number of animals which had convulsions by 55%. L-a-methyldopa did not affect the 24

Table XXIX. Convulsive latency time (CLT), 24 hour mortality and hepatic glycogen, G6P and PEPCK activity, two hours post-treatment in 60 day old male rats given 1 LD50 HEOD and either, 200 mg/kg i.p. L-a-methyl-dopa (treated) or acid-saline (control) at 0 and 6 hours after HEOD. Tabled values represent mean \pm SE.

A: 24 hour data.

| | CONTROL GROUP | | TREATED GROUP | | t or χ^2 |
|-------------------|-------------------|-------------------|---------------|------------------|--|
| | N rats | Mean \pm SE | N rats | Mean \pm SE | |
| CLT minutes | 9/10 ¹ | 400.0 \pm 157.8 | 4/10 | 157.0 \pm 42.5 | 1.489 |
| 24 hour mortality | 7/10 | 70.0% | 6/10 | 60.0% | 0.833 ² 0.202 ³ |

B. 2 hour data.
(n=5 rats/group)

| | CONTROL Mean \pm SE | TREATED Mean \pm SE | t |
|--|--------------------------|--------------------------|---------|
| Hepatic glycogen ug/mg wet weight | 18.39 \pm 4.01 | 16.12 \pm 3.15 | 0.443 |
| Hepatic PEPCK activity Unit/g wet weight | 1.19 \pm 0.060 | 0.61 \pm 0.114 | 4.507 b |
| Hepatic G6P activity Unit/g wet weight | 9.41 \pm 0.852 | 10.91 \pm 0.114 | 1.662 |

1 : sample size is total number of convulsive rats/group

2 : χ^2 value for mortality in control group versus expected mortality for 1 LD50 HEOD

3 : χ^2 value for mortality in treated group versus mortality in control group

b : P0.01

hour mortality produced by HEOD, nor did it influence hepatic G6P activity, neither did it alter hepatic glycogen content (Table XXIX). However, hepatic PEPCK activity at two hours was significantly decreased in the HEOD and L-a-methyldopa-treated rat. This latter result suggests that HEOD stimulation of gluconeogenesis was impaired in these animals.

In contrast to atropine, the antihyperglycemic effect of L-a-methyldopa may be reimposed, since the second dose of the drug caused a 24% decrease in the plasma glucose level (i.e. from 147 mg/dl to 112.5 mg/dl). This decrease was not of sufficient magnitude to be statistically significant. However, this lack of significance may result from the large variance in the data of the control animals. In addition, both of the L-a-methyldopa injections provoked an equivalent reduction in plasma glucose concentration (i.e. about 25%) and in both instances, the effect endured for an equal period of time (i.e. two hours). Thus L-a-methyldopa can consistently, albeit briefly, inhibit the HEOD-induced carbohydrate response.

Previously the suppression of HEOD-induced hyperglycemia by phenobarbital or atropine was accompanied by a reduction in the acute toxicity of dieldrin. Therefore, one might expect decreased mortality in the HEOD and L-a-methyldopa-treated rat. However, this was not found to be the case (Table XXIX).

While the 24 hour HEOD-induced mortality was unaffected by L-a-methyldopa, there is reason to believe that the drug did delay the onset of the mortality produced by dieldrin.

No further mortality occurred in the controls after nine hours had elapsed while the majority of the mortality (66%) seen in the treated group occurred after that time. Finally, in view of the brief duration of the antihyperglycemic effect caused by the drug, it is perhaps not surprising that it was unable to attenuate the 24 hour toxicity of HEOD.

The mortality in the HEOD and L-a-methyldopa-treated rats occurred only during those times of the experiment when the plasma glucose levels of treated and control rats were equivalent (i.e. 4 to 6 and 9 to 24 hours). No mortality occurred in the treated group during periods where the plasma glucose level of these animals was less than that of the controls (i.e. 0 to 2 and 6 to 8 hours). Thus, during the periods in which L-a-methyldopa suppressed HEOD-induced hyperglycemia, HEOD-induced mortality was also reduced.

The time of onset of convulsions was not altered by the drug (Table XXIX). However, L-a-methyldopa did reduce the number of animals which had convulsions by 55%. Consequently, the drug was effective in blocking HEOD-induced CNS hyperstimulation in only some of the rats of the treated group.

Convulsions began in the treated rats at about the same time (two and one-half hours) that the anti-hyperglycemic effect of L-a-methyldopa terminated (two hours). This result is consistent with the conclusion that the HEOD-induced hyperglycemic response is mediated by the CNS. Thus when convulsive activity was absent in the treated group, no hyperglycemia was evoked by dieldrin.

Since L-a-methyldopa can block the HEOD-induced carbohydrate response, sympathetic nervous activity must play some role in the maintenance of the response. Sympathetic stimulation of the pancreas is known to inhibit the release of insulin (Smith & Porte, 1976). Thus the antihyperglycemic effect of the drug may result from an increased release of pancreatic insulin.

The brevity of the antihyperglycemic effect of L-a-methyldopa relates to the relatively short duration of action of this drug. In the rat, peak L-a-methyldopa concentration in the brain occurs within one hour after intraperitoneal injection (Uretsky & Seiden, 1969). These authors also state that the biological half-life at this site is only 2.4 hours. In man, the plasma half-life of the drug is only 1.8 hours (Kwan et al., 1976). These half-lives correlate well with the observed period of the antihyperglycemic effect.

L. d-l-Propranolol: effects in HEOD treated adult rats.

L-a-methyldopa inhibits activity in the sympathetic nervous system by causing the synthesis of a "false neurotransmitter" (Weiner, 1980) and also blocks sympathetic outflow from the CNS (Henning, 1969). Thus with L-a-methyldopa treatment, both α and β -adrenergic activity is effectively inhibited, at the level of the pancreas.

A decrease in β -adrenergic activity would be expected to result in a decrease in the release of glucagon (Smith & Porte, 1976; Unger et al., 1978). If sympathetically mediated glucagon release was responsible for the hyperglycemia produced in

the HEOD-treated rat, then one should be able to eliminate or at least attenuate the hyperglycemia by administering a β -adrenergic blocking agent. This hypothesis was tested by giving d,l-propranolol to the HEOD-exposed animal.

Adult male rats were given 1 LD50 HEOD and at the times indicated in Figure 12, 8 mg/kg s.c. d,l-propranolol (treated). Control rats received HEOD and 0.9% saline at those times.

Both experimental groups exhibited essentially identical plasma glucose patterns throughout the experiment (Figure 12). A statistically significant difference in the plasma glucose level between the two groups was detected only at one hour after HEOD administration. At that time, the plasma glucose level in the HEOD and propranolol-treated rat was reduced by 27%. Thus, propranolol produced a small and physiologically insignificant delay in the onset of HEOD-induced hyperglycemia.

Table XXX shows that propranolol did not alter the 24 hour mortality, the time of onset of convulsions, the hepatic PEPCK activity or the hepatic glycogen content in the HEOD-treated rat. The plasma half-life of propranolol is 108 minutes in the rat (Schneck et al., 1977) and a dose of 2 mg/kg i.p. will completely block the adrenergic-mediated pressor response to 0.3 μ g/kg i.v. norepinephrine in the adult rat (Weiss et al., 1976). Therefore, the lack of significant effects in the HEOD-exposed rat cannot be attributed to either an insufficient dose of propranolol or too short a duration of action of the drug. Thus we conclude that β -adrenergic activity plays no role in the effects normally elicited by dieldrin in the adult?

Figure 12. Mean plasma glucose (mg/kg) in 60 day old male rats given 4 LD50 HEOD and either 8 mg/kg s.c. d,l-propranolol (treated) or 0.9% saline (control). The figure also presents, for the two groups, the percent mortality and the significance of plasma glucose differences.

ns: not significant

xx: $P < 0.01$

n: 12 rats (control)

n: 12 rats (treated)

| TIME hours | pre | 0 | 0.5 | 1 | 1.5 | 2 | 3 | 4 | 5 | 6 |
|------------|-----|----|-----|----|-----|----|----|----|----|----|
| CONTROL % | 0 | 0 | 0 | 0 | 0 | 0 | 8 | 8 | 8 | 33 |
| TREATED % | 0 | 0 | 0 | 0 | 0 | 0 | 29 | 41 | 41 | 47 |
| P. GLUCOSE | ns | ns | ns | ** | ns | ns | ns | ns | ns | ns |

| TIME hours | 6.5 | 7 | 7.5 | 8 | 9 | 10 | 11 | 12 | 18 | 24 |
|------------|-----|----|-----|----|----|----|----|----|----|----|
| CONTROL % | 58 | 66 | 66 | 66 | 66 | 66 | 66 | 66 | 66 | 66 |
| TREATED % | 53 | 53 | 53 | 53 | 59 | 65 | 65 | 65 | 65 | 65 |
| P. GLUCOSE | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |

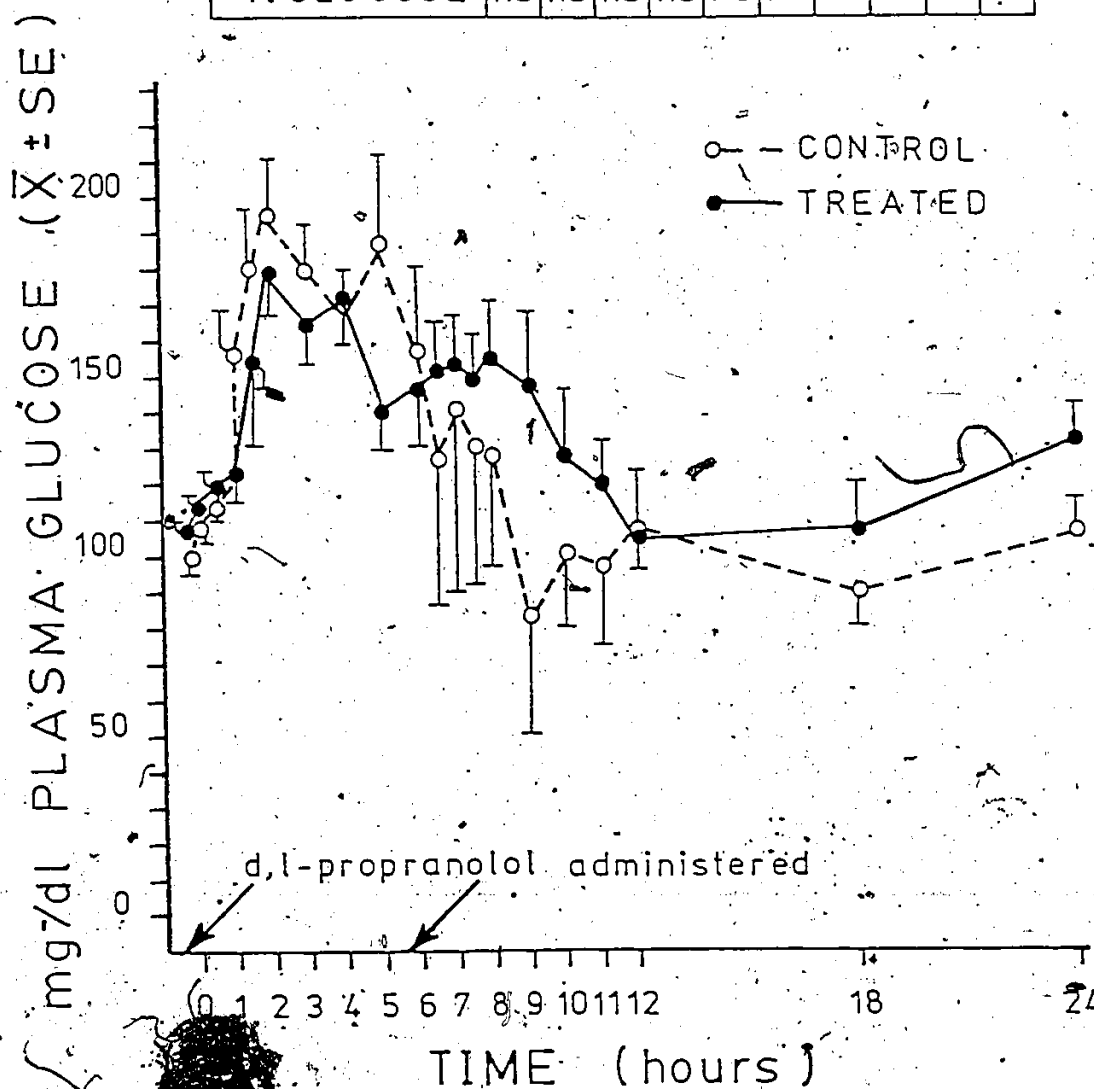


Table XXX. Convulsion latency time (CLT), 24 hour mortality and hepatic glycogen and PEPCK activity, two hours post-treatment in 60 day old male rats given 1 LD50 HEOD and either, 8 mg/kg s.c. propranolol (treated) or 0.9% saline (control) at 15 minutes prior to, and 5 and 3/4 hours after HEOD. Tabled values represent mean \pm SE.

| A. 24 hour data. | | | | | |
|----------------------|--------------------|------------------|---------------|------------------|--|
| | CONTROL GROUP | | TREATED GROUP | | |
| | N rats | Mean \pm SE | N rats | Mean \pm SE | t or χ^2 |
| CLT minutes | 11/12 ¹ | 121.8 \pm 13.8 | 13/17 | 143.0 \pm 22.1 | 0.813 |
| 24 hour mortality | 8/12 | 66.8% | 11/17 | 64.7% | 0.171 ² 0.271 ³ |

| B. 2 hour data (n=4 rats/group) | | | |
|--|--------------------------|--------------------------|-------|
| | CONTROL Mean \pm SE | TREATED Mean \pm SE | t |
| Hepatic glycogen ug/mg wet weight | 12.9 \pm 4.10 | 10.91 \pm 3.13 | 0.386 |
| Hepatic PEPCK activity Unit/g wet weight | 0.78 \pm 0.080 | 0.81 \pm 0.026 | 0.353 |

1 : sample size is total number of convulsive rats/group

2 : χ^2 value for mortality in control group versus expected mortality for 1 LD50 HEOD

3 : χ^2 value for mortality in treated group versus expected mortality for 1 LD50 HEOD

rat.

However, it does not follow that α -adrenergic activity does not play a role in the HEOD-induced carbohydrate response. When all sympathetic activity was inhibited in the HEOD-exposed rat, by L- α -methyldopa treatment, hyperglycemia did not occur. By inference then, only α -adrenergic stimulation is involved in the response. Thus it would appear that the HEOD-induced carbohydrate response develops as a consequence of increased parasympathetic and α -adrenergic activity.

M. Summary of results.

The following is a brief point form summary of the results found in this study.

Mi. Acute toxicity of dieldrin in the three age groups.

1. Dieldrin LD50 values in the three ages when ranked from lowest to highest were: 10 < 5 < 60 day olds.

Thus the acute toxicity of HEOD varies with age.

Mii. Effects of dieldrin on carbohydrate metabolism in five and ten day olds.

1. Dieldrin briefly induced hyperglycemia only in the five day old, one hour after administration.
2. Dieldrin decreased plasma free fatty acid concentration only in the five day old, at one hour.
3. Dieldrin decreased hepatic glycogen content in both ages at one hour only.
4. Hepatic fructose-1,6-diphosphatase activity was increased by dieldrin in the five day old at one hour only and was decreased in the ten day old at three hours only.
5. Dieldrin increased hepatic PEPCK activity in both ages at three hours only.

The above results indicate that dieldrin does not consis-

tently alter carbohydrate metabolism in the immature rat.

6. Glucose attenuates HEOD-induced mortality in ten day olds.

This suggests that the HEOD-hyperglycemic response may have adaptive value in young rats in which it occurs.

Miii. Effects of dieldrin on carbohydrate metabolism in the adult male rat.

1. Dieldrin caused pronounced hyperglycemia in 60, 120 and 200 day old males.
2. Dieldrin increased hepatic PEPCK activity at one and three hours after administration to the 60 day old.

Thus dieldrin consistently induces a hyperglycemic/glucogenic response in the adult rat.

3. Increasing the hyperglycemia in the dieldrin-exposed rat with d-glucose did not increase HEOD-induced mortality.

Thus enhancing the "normal" HEOD-induced hyperglycemia does not alter the acute toxicity of dieldrin.

4. 2-Deoxyglucose increased hyperglycemia and by inference therefore decreased the CNS intracellular glucose concentration in the HEOD-exposed adult rat. It did not change the latency to convulsions or the acute toxicity of HEOD.

Therefore, decreasing the CNS intracellular glucose concentration had no effect on HEOD-induced CNS hyperstimulation or toxicity. This suggests that the CNS events elicited by dieldrin may occur independent of HEOD-induced hyperglycemia.

5. Insulin not only prevented HEOD-induced hyperglycemia but also caused hypoglycemia. Insulin did not prevent the HEOD-induced increase in hepatic PEPCK activity at two hours. However, the hormone decreased the latency to convulsions and increased the mortality caused by dieldrin.

These results indicate that HEOD-induced gluconeogenesis was initiated in the HEOD and insulin-treated rat but that insulin prevented the hyperglycemic expression of this effect. Thus, abolishing HEOD-induced hyperglycemia and installing hypoglycemia may have increased CNS hyperactivity and the acute toxicity of dieldrin.

6. Phenobarbital attenuated the hyperglycemia and virtually eliminated the convulsions and the mortality caused by dieldrin.

Therefore, CNS hyperstimulation is responsible for the mortality and the hyperglycemia induced by dieldrin.

7. d-Glucose restored, the hyperglycemia and the mortality in the HEOD and phenobarbital-treated rat, to levels normally found in rats given only dieldrin.

Thus, the HEOD-induced hyperglycemic response may be toxic.

8. Insulin in the HEOD and phenobarbital-treated rat produced a hypoglycemia of greater magnitude than that found in the rat given only HEOD and insulin. However, the latency to convulsions and the mortality in the HEOD and phenobarbital and insulin-treated rat was less than that in the HEOD and insulin-treated rat. Hepatic PEPCK was not elevated in the HEOD, phenobarbital and insulin-exposed rat.

Therefore, phenobarbital inhibits HEOD-induced gluconeogenesis and insulin enhancement of HEOD-induced CNS hyperstimulation. These effects result in a decrease in HEOD-induced mortality which would otherwise be elevated by insulin. Since hypoglycemia was increased but mortality was decreased, the HEOD-induced hyperglycemic response may lack adaptive value.

9. Atropine prevented HEOD-induced hyperglycemia for four hours only. Atropine also increased the latency to convulsions and reduced the mortality caused by dieldrin. Simultaneous onset of convulsions and hyperglycemia occurred at about four hours. Hyperglycemia reached peak levels at six hours and mortality began to occur at seven hours.

Contrary to previous experiments, the above results suggest that HEOD-induced CNS hyperstimulation may not be lethal in the absence of the fully developed HEOD-induced hyperglycemic response. Further, since atropine initially blocks the HEOD-induced hyperglycemic response, the parasympathetic nervous system is clearly involved in the response.

10. L-a-Methyldopa in the dieldrin-exposed rat decreased the plasma glucose concentration by 25%. This effect endured for about two hours and was reproducible with subsequent L-a-methyldopa injections. No mortality occurred during the periods of reduced hyperglycemia. However, the drug did not reduce the HEOD-induced 24 hour mortality.

Thus, the sympathetic nervous system also plays a role in the HEOD-induced hyperglycemic response. However, this role is probably minor, since the antihyperglycemic effect of L-a-methyldopa is small. These results also

suggest that hyperglycemia is a required component of the acute toxicity of dieldrin.

11. d-1-Propranolol in the dieldrin-treated rat had no effect on the HEOD-induced hyperglycemic response or on the acute toxicity of the pesticide.

Thus, β -adrenergic mediated activity plays no role in the etiology of the HEOD-induced carbohydrate response.

Table XXXI presents a summary of the effects on HEOD-induced hyperglycemia, CNS hyperstimulation and mortality found in the above experiments.

Table XXXI. Summary of effects produced on HEOD-induced hyperglycemia, CNS hyperactivity (convulsion latency time) and mortality (24 hour) in experiments performed on 60 day old male rats. All effects are relative to "normal" situation seen in rats given only HEOD.

| Experimental condition (HEOD ¹ and;) | Effect produced on HEOD-induced | | |
|--|---------------------------------|-------------------|-----------|
| | Hyperglycemia | CNS hyperactivity | Mortality |
| d-glucose | mk. inc. | N/R | N/C |
| 2-deoxyglucose | mk. inc. | N/C | N/C |
| insulin | mk. dec. | mk. inc. | mk. inc. |
| phenobarbital | dec. | mk. dec. | mk. dec. |
| phenobarbital and d-glucose | N/C | dec. | N/C |
| phenobarbital and insulin | mk. dec. | N/C | N/C |
| atropine | tr. dec. | mk. dec. | mk. dec. |
| l-a-methyldopa | tr. dec. | N/C | N/C |
| d,l-propranolol | N/C | N/C | N/C |

N/R : not recorded

mk. : markedly

inc. : increased

1 : HEOD dose equalled 1 LD50 in all cases.

N/C : not changed

tr. : transitory

dec. : decreased

DISCUSSION

A. Acute Toxicity of HEOD administered per os.

The acute toxicity of HEOD was found to vary with the age of the animal. In this respect, the 10 day old pup was 1.5 to 2.5 fold more sensitive than either the five day old or the adult, respectively.

In the cases of the 10 day old and the adult, our LD50's for HEOD agree with the literature (Hodge et al., 1967; Lu et al., 1965). An LD50 for the five day old rat has not previously been reported. However, the literature supports our conclusion that the five day old is more resistant to HEOD than is the 10 day old. Lu et al. (1965) found the LD50 value for HEOD in the one day old rat (167.8 mg/kg) to be 7 fold greater than that in the "pre-weaning rat" (ie. 10 to 14 day old) and 4.5 fold greater than that in the adult.

Increased resistance to the toxicity of DDT in the neonate has also been reported (Lu et al., 1965; Henderson & Woolley, 1969). In fact, a low responsiveness to CNS stimulants appears to be a general attribute of the young mammal. For example, Goldenthal (1971) found the LD50 values for d-amphetamine, picrotoxin and strychnine were all significantly greater in the newborn than in the adult rat. This increased resistance to CNS stimulants has been suggested to result from the immature development of the CNS in the neonate (Henderson & Woolley, 1965). Thus, Pylkko & Woodbury (1961) found that rats aged one to four days were unable to produce

full clonic or tonic convulsions even when given lethal doses of strychnine. Furthermore, these authors found that the CD50 (convulsive dose for 50% of rats treated) did not change until the animals reached day eight of age, the age at which the CNS began to mature. Typical adult CD50's for strychnine were not found until the rats were 12 to 16 days old.

Henderson & Woolley (1969) found that the LD50 for pp-DDT in 10 day old rats was 728 mg/kg compared to 250 mg/kg in the 60 day old adult. This increased LD50 was inversely correlated with significantly lower DDT concentrations in liver, brain and spinal cord of the younger animals. Since the organochlorines accumulate in body fats, the lower concentrations were explained on the basis of a lower lipid content in these organs in the young rats. However, reduced lipid content probably does not solely account for the increased resistance to pp-DDT seen in the young rats. This inference is drawn from the fact that the pp-DDT concentration in brain lipids of young rats who died, when expressed as $\mu\text{g/g}$ of lipid rather than as $\mu\text{g/g}$ of tissue weight, was actually four fold greater than that found in brain lipids of adult animals who died.

B. Effects of d-glucose on the acute toxicity of HEOD in the immature rat.

In addition to being the most sensitive to the acute toxicity of HEOD, the 10 day old did not exhibit a hyperglycemic response to HEOD treatment. These results suggest that hyperglycemia might confer resistance to the acute toxicity of the

pesticide. This was confirmed when it was found that the administration of glucose to the HEOD-exposed 10 day old rat attenuated the toxicity of HEOD. Thus an elevated blood glucose concentration has protective value to the HEOD-exposed 10 day old rat.

However, this protective effect was not seen in the adult HEOD-treated rat. Nor is the administration of glucose of any adaptive value in the DDT-exposed rabbit (Stohlman & Lillie, 1948). An "antidote" is any agent which acts to limit the intensity, or reverses the effect(s) of a toxic compound (Loomis, 1978). Thus, glucose, as an antidote to the toxicity of chlorinated hydrocarbon pesticides, is age-dependant in the rat.

In order for a toxic compound to elicit an effect, the compound must have free access to its effector site(s) and a sufficient quantity must be present at that effector site(s). Antidotes typically counteract toxicity therefore, by interfering, in some manner, with this distribution. For example, any process or agent which acts to enhance either the rate of elimination or the biotransformation of a toxic compound into inactive metabolites, will reduce the concentration of the compound at its effector site(s).

It is highly unlikely that either increased elimination or biotransformation were responsible for the decreased toxicity of HEOD seen in the glucose-treated 10 day old pup.

In addition to an immature CNS, the young animal also possesses a poorly developed hepatic monooxygenase system.

Several authors have reported that both the amount and the activity of the mixed-function oxygenase system enzymes are low in neonatal rats. For example, the concentration of cytochrome P450 in the liver of 10 day old rats is approximately one-half that of adult liver (Acosta et al., 1979). In the Wistar rat, the concentration of hepatic P450 increases steadily after six days of age to reach a peak level by 30 days of age (Basu et al., 1971). The activities of; umbelliferone glucuronyl transferase, p-nitrobenzoate reductase, biphenyl-2-hydroxylase and biphenyl-4-hydroxylase are also low in the immature rat and do not increase to adult levels until 25 to 30 days of age (Basu et al., 1971). Finally, the rate of transformation of DDT to DDE and DDD is significantly less in the 10 day old rat than in the adult (Henderson & Woolley, 1969).

Dieldrin, when administered to adult mammals is biotransformed by the liver into several metabolites (Matthews et al., 1971; McKinney et al., 1972; Lindstrom et al., 1974; Chipman & Walker, 1979). There exist species differences in the type, number and rate of metabolite formation from dieldrin (Matthews et al., 1971; Hutson, 1976). However, the major metabolite of dieldrin formed and, according to Matthews & McKinney (1974), the only metabolite known to be excreted in significant quantity in all higher animals (i.e. birds and mammals) is 6,7-trans-aldrindihydrodiol.

Several authors have postulated that it is this metabolite which is actually responsible for the toxic effects of

dieldrin in the American cockroach, (Wang et al., 1971) amphibian (Akkermans et al., 1973, 1975a, 1975b) and cephalopod mollusk (Van den Bercken & Narahashi, 1974). However, Schroeder et al. (1977) found that while both the cis and the trans isomers of aldrindihydrodiol readily enter the CNS of the American cockroach, they are less than 0.01 fold as toxic as dieldrin is to this insect. Furthermore, although the toxicity of trans-aldrindihydrodiol has not been extensively studied in the mammal, all reports to date indicate that this dieldrin metabolite is not responsible for the toxicity of the insecticide. Thus, Korte & Arent (1965) found that in adult mice, the LD50 for trans-aldrindihydrodiol was 10 fold greater than that of HEOD itself. Furthermore, Joy (1977) reported that in the adult cat, dieldrin, at a dose of 2 to 4 mg/kg (i.v.) induced convulsions while trans-aldrindihydrodiol administered at doses of 20 mg/kg neither provoked convulsions nor altered the electroencephalograph pattern in the cat. It seems evident therefore that in the mammal, dieldrin acts directly on the CNS and biotransformation to trans-aldrindihydrodiol is not a pre-requisite for toxicity. Consequently, in the adult mammal, the processes of biotransformation should reduce rather than increase the toxicity of HEOD.

Viewed in this light, the relatively non-functional monooxygenase system of the immature rat might actually enhance the toxicity of dieldrin. However, this would occur only if the CNS was sufficiently mature to allow it to respond to

HEOD stimulation.

In the five day old rat, both the CNS and the mixed-function oxygenase (MFO) system are immature. Thus the five day old probably can not detoxify HEOD to any significant extent. However, since the CNS of this animal is also poorly developed, high levels of HEOD would be required to elicit mortality. In the adult rat, on the other hand, both systems are fully developed.

In the 10 day old, the CNS is mature, at least insofar as its ability to undergo "adult type" convulsions (Pylkko & Woodbury, 1961). However, the MFO system is not mature (Henderson & Woolley, 1969; Basu et al., 1971). Thus the enhanced sensitivity of the 10 day old rat to HEOD may result as a consequence of it possessing a relatively mature CNS but essentially non-functional MFO system.

In regards to the possibility of enhanced HEOD elimination in the glucose treated 10 day old pup, it is unlikely that this would explain the antidotal quality of glucose. The elimination of water soluble compounds is often enhanced by inducing diuresis in vivo. However, dieldrin is highly lipophilic, so an increase in urine flow rate would not enhance the excretion of this compound.

Furthermore, according to Boyd (1973) the presence of a high glucose concentration in the gut tends to osmotically attract body fluids into the lumen of the gastrointestinal tract. This effect produces diarrhea and reduces urine output by producing hypovolemia in the animal. Neither of these

latter effects were observed in either the 10 day old or the adult rat treated with glucose and HEOD.

Finally, if the elimination rate of HEOD had in fact been increased in the 10 day old glucose-treated pups, it is difficult to explain how an effectively reduced dose of dieldrin could still cause a mortality level of 50%, once the administration of glucose was discontinued. Thus it is highly improbable that the total body-burden of HEOD present in the 10 day old pups was reduced to any significant extent by glucose treatment.

Rather, there is reason to believe that glucose indirectly and temporarily denied dieldrin access to the CNS of these 10 day old pups. However, prior to presenting a proposed mechanism to explain the glucose effect, a brief review of the pharmacokinetics of HEOD and of the lipid metabolism of the suckling rat is required.

Heath & Vandekar (1964) extensively studied the toxicity, metabolism and distribution dynamics of [^{14}C]-labelled HEOD administered to the adult rat as a single per os dose dissolved in arachis oil. These authors found that HEOD was readily absorbed from the stomach and small intestine into the portal vein and not the thoracic duct. The absorbed dieldrin was transported in the blood bound to lipoproteins and was rapidly redistributed into organs with a high lipid content (e.g. adipose tissue). Thus HEOD, like all other organochlorines, is distributed in the body on the basis of its lipophilic nature. It is not surprising therefore that

adipose tissue is the main site of HEOD disposition. Hayes (1974) for example, found that after a single oral dose of HEOD to the adult rat, the HEOD content of body depot fat was two orders of magnitude greater than that of brain.

In the young rat, HEOD also distributes on the basis of organ lipid content. However, the plasma concentration of organochlorine pesticides is much greater in the suckling rat than in the adult. Henderson & Woolley (1969) found that the plasma concentration of pp'DDT in the 10 day old rat was two fold greater than that in the adult during the first eight hours following dosing. Brain pp'DDT concentration in these young rats was initially two fold less than that found in the adults. However, peak brain DDT level in both ages occurred four hours after exposure. Hayes (1974) also found that in the adult rat given HEOD, peak brain concentration did not occur until four hours after dosing. Henderson & Woolley (1969) explained the differences in pp'DDT distribution found between their 10 day old and adult rats on the basis of the lower tissue lipid content and the higher plasma lipid content in the 10 day olds. Thus the 10 day old possesses two reservoirs for the storage of chlorinated hydrocarbon insecticides: the adipose tissues and the blood lipids.

Once organochlorine pesticides are sequestered into depot fat, their level in this tissue tends to remain relatively static. However, severe conditions which mobilize depot fat stores, such as starvation, also cause a net release of stored pesticides (FitzHugh & Nelson, 1947; St. Omer, 1978). Baron &

Walton (1971) have stated that the mobilization of fat is generally accompanied by a marked elevation in the concentration of blood lipids. Thus plasma HEOD concentration will also rise provided it is released from fat depots.

It is also interesting to note that different tissues mobilize HEOD at different rates. Robinson et al. (1969) found that in the adult rat, brain HEOD levels decayed in an exponential fashion (e.g. HEOD half-life in brain was three days) while a steady linear decay was seen in adipose tissue (e.g. HEOD half-life in adipose was 10 days). Therefore, it is possible that HEOD might actually be "cleared" from the brain, under conditions of metabolic substrate insufficiency prior to its complete removal from depot fat.

Bearing these observations in mind, let us now briefly examine lipid metabolism in the suckling rat.

The composition of rat milk is 69% lipid, 23% protein and 8% carbohydrate (Beaudry et al., 1977). The protein content of rat milk is of minor consequence to the energy requirements of the suckling rat, since only 4% of the basal calories supplied are derived from protein catabolism (Gil-Ad et al., 1975). Thus the suckling rat is generally considered to be both dependant on, and adapted to, lipid metabolism for its nutritional needs.

According to Hahn (1970) this predominance of lipid metabolism in the young rat produces several effects. For example, plasma concentrations of free fatty acids, glycerol and triglycerides (present as lipoprotein-bound chylomicrons)

are all typically much higher in the suckling rat than in either the fetal or the adult rat.

In the liver of the suckling rat, fatty acid oxidation is increased and fatty acid and cholesterol synthesis are suppressed. This enhanced fatty acid oxidation results in an extremely high production rate of ketone bodies, and these ketones supply the energy requirements of the animal (Hahn, 1970). It has been shown that, in the brain of the suckling rat, ketone bodies provide the energy requirements and also serve as the major source of carbon for biosynthetic processes (Stumpf & Kraus, 1979). In fact glucose is not normally utilized as an energy source in the brain of these animals, but rather is used in the pentose phosphate shunt pathway to provide NADPH for use in the synthesis of myelin lipids.

The catabolism of dietary fatty acids and triglycerides provides a large surplus of both glycerol and acyl CoA which in turn act to stimulate gluconeogenesis and inhibit glycogenolysis in the liver (Hahn, 1970). Enhanced gluconeogenesis in the suckling rat is reflected by the activity of hepatic fructose-1,6 -diphosphatase which attains a maximal value by eight to ten days of age, and thereafter decreases to reach the adult level of activity by 25 to 30 days of age (Taylor et al., 1967).

A recent study by Turlan et al. (1983) demonstrates the dependance of the neonatal rat on fatty acid oxidation to maintain gluconeogenesis. These authors found that when long chain fatty acid oxidation in the one day old suckling rat was

inhibited by 2-tetradecylglycidic acid (McN-3716), both hypoketonemia and hypoglycemia developed within six hours. Also the rate of hepatic gluconeogenesis from U-[14 C]-lactate was reduced five fold, three hours after McN-3716 administration. These effects were all promptly reversed (i.e. within three hours) following feeding of medium chain-length triglycerides to the neonates.

Pegorier et al. (1983) also found a five fold increase in the rate of incorporation of U-[14 C]-lactate into glucose in 16 hour old rat pups fed medium chain-length triglycerides, when compared to fasted controls. Furthermore, these authors showed that 3-mercaptopycolinate (a gluconeogenic inhibitor) could completely suppress the hyperglycemia which was normally induced by medium chain-length triglyceride feeding. Finally, it is interesting to note that glycerol feeding, although greatly elevating blood glycerol levels (i.e. 14 to 17 fold), only produced a moderate 40% increase in blood glucose level.

In the adipose tissue of the suckling rat, the catabolism of endogenous triglyceride is effectively inhibited since the activity of "hormone-sensitive lipase" is normally low (Hahn et al., 1968; Hahn, 1970). On the other hand, adipose tissue, as well as heart and lung, exhibit high rates of catabolism of dietary triglyceride since the activity of "lipoprotein lipase" is elevated (Hahn, 1970). Thus the conversion of dietary fat into *de novo* depot triglyceride proceeds at a high rate.

It is well known that a high glucose diet stimulates triglyceride synthesis in the adult rat (Fallon & Kemp, 1968;

Merkens et al., 1980). However to our knowledge, the *in vivo* effects of glucose administration on lipid synthesis in the suckling rat have not been investigated. Furthermore, relatively few studies have examined the *in vitro* effects of glucose on lipid synthesis in this animal.

Hahn et al. (1968) studied the *in vitro* incorporation of [^{14}C]-labelled glucose, acetate and pyruvate into the triglycerides of white and brown adipose tissue obtained from fetal, suckling and adult rats. All three compounds were incorporated into adipose triglycerides of rats of all ages. However, glucose was an inferior precursor, when compared to either acetate or pyruvate, for triglyceride synthesis in suckling rat adipose tissue.

Ballard & Hanson (1967) studied the *in vitro* effects of [^{14}C]-labelled glucose and acetate on lipid synthesis in liver slices obtained from fetal, suckling and adult rats. It was found that lipogenesis in liver tissue from either precursor was extremely low in the suckling rat. This result has recently been confirmed by Madvig & Abraham (1980) who found the *in vitro* rate of fatty acid synthesis from [$1\text{-}^{14}\text{C}$]-glucose or [$6\text{-}^{14}\text{C}$]-glucose in the liver of the 16 day old rat to be only 5% of the rates found in the livers of either the fetal or the weanling (i.e. 21 day old) rat.

However, Ballard & Hanson (1967) also found that increasing the concentration of [^{14}C]-labelled glucose from 1 to 100 mM in their incubates containing suckling rat liver caused a 35 fold increase in the amount of isotope incorporated into

fatty acid. Thus there is reason to believe that in the liver of the suckling rat, lipid synthesis which is normally suppressed, as a consequence of the high fat diet, can be stimulated by the presence of a high intracellular glucose concentration.

This latter result provides the basis for a hypothesis that explains how glucose prevented the toxicity of HEOD in the 10 day old pup. In our young rats, HEOD would be absorbed and distributed in the body on the basis of the lipid content of the tissues. Thus one would expect to find high levels of HEOD in both the plasma, bound to lipoproteins, and in the depot fat, bound to triglycerides. The brains of these animals would have a lower lipid content and would be expected to have a lower HEOD concentration than either the blood or the adipose tissue (Robinson et al., 1969).

Fasting in the control (HEOD, non-glucose treated) rats would have ended the supply of dietary lipid. Since the suckling rat is adapted to, and dependant on, lipid for its energy requirements, fasting would also cause the mobilization of depot fat. The HEOD which had been distributed into that fat would also be mobilized, so that the lipid-rich blood of the fasted pup would also contain a high HEOD concentration. Thus the fasted 10 day old rat would lose an important reservoir, the adipose tissue, for HEOD containment, which in the fed pup would serve to lower the amount available to the CNS. Therefore, it is possible that as a result of the redistribution of HEOD caused by fasting, the concentration of HEOD in both the blood and brain of these pups, if not actually

increased, might at least remain at a relatively constant high level throughout the 48 hour experimental period.

In the glucose-treated pups (HEOD and glucose) in contrast, it is possible that glucose activated the hepatic lipogenic pathway and thus negated the loss of dietary lipid. Perhaps therefore, glucose ensures that the net export of lipid from the liver to the adipose tissue still occurs in these pups. Thus with glucose the concentration of blood lipids may remain at a level sufficient to allow de novo triglyceride synthesis to continue in the adipose tissue. This postulated continuation of de novo synthesis should have two effects. First, new depot fat would be laid down, and thus the concentration of HEOD present in the blood, bound to lipoproteins would decrease as it was redistributed into this new fat. Secondly, the decreased HEOD concentration in the blood may shift the equilibrium in favour of HEOD moving from the CNS to the plasma. Consequently the HEOD concentration in the CNS should decrease. Thus with glucose, the HEOD-burden of the CNS might be reduced at the expense of an increase in the HEOD concentration of depot fat. Under these conditions the 10 day old rat would be protected against the toxic effects of dieldrin. However, once glucose administration had ceased, and fasting had been reinstated, the process would reverse itself and the HEOD sequestered in depot fat would then be liberated into the blood and thence redistribute back into the CNS, to ultimately produce toxicity. This scheme would explain why the lethal effects of HEOD were only delayed and not eliminated in the HEOD and glucose treated

10 day old rat.

C. Carbohydrate metabolism in the immature rat - Effects of HEOD.

HEOD did not consistently alter the carbohydrate metabolism of the young rat (5 and 10 day old). For example, HEOD, one hour after administration to the five day old increased plasma glucose concentration and hepatic FDP activity and decreased plasma FFA and hepatic glycogen levels. However, these effects were only transitory, since three hours after HEOD treatment, these parameters had all returned to normal. Dieldrin (1 LD50) decreased hepatic glycogen levels at one hour after administration to the 10 day old rat. However, by three hours after HEOD, hepatic glycogen had returned to normal and hepatic FDP activity was reduced. In both five and ten day olds, HEOD caused an elevation in hepatic PEPCK activity three hours after treatment.

These results indicate that HEOD causes a brief period of increased glycogenolysis in the young rat and perhaps a brief and transitory period of hyperglycemia. Finally HEOD is effective in causing an elevation in the activity of hepatic PEPCK in the young rat. However this latter effect occurs late (i.e. at three hours after exposure to the pesticide) and is not accompanied by hyperglycemia.

Thus it seems clear that HEOD does not evoke either a pronounced or a long-lived carbohydrate response in the young rat. We have seen that exogenous glucose in the 10 day old rat is an effective antidote with regard to the acute toxicity

of HEOD. Therefore, since an HEOD-induced carbohydrate response may be of adaptive value to the young rat, why is this animal apparently unable to exploit this potentially adaptive response?

In order for a pharmacological response to become apparent, it must persist for a sufficient period of time for a physiological effect to be produced (Loomis, 1978). The above results suggest that the HEOD-induced carbohydrate response may be initiated. Yet it is evident that the response does not persist. Therefore it would seem informative to examine those factors which prevent the full development of the HEOD-induced carbohydrate response.

The potential for HEOD-induced gluconeogenesis and therefore increased activities of the hepatic gluconeogenic enzymes may exist in the young rat. For example, the activities of hepatic FDP, PEPCK and pyruvate carboxylase are all higher (by about 25%) in the 10 day old than in the adult rat (Ballard, 1970). Gluconeogenesis from amino acid precursors reaches a peak level in the five day old which is 2.5 fold greater than that found in the adult. This effect is due to the increased activity of hepatic transaminases. The activity of hepatic aspartate transaminase, for example, is two fold greater in the 10 day old rat than in the adult (Yeung & Oliver, 1967). The activity of hepatic G6P reaches a peak level in the two day old rat which is 100% greater than that of the adult; it then declines to reach the adult level by 10 days of age (Greengard, 1969).

If the activities of these gluconeogenic enzymes were at near maximal levels, a condition which fasting should presumably impose, then HEOD would not be able to cause further elevation in their activity. Thus HEOD would fail to initiate a carbohydrate response. However, this in fact seems unlikely. An HEOD-induced carbohydrate response was attempted in the five day old rat and in both the five and the ten day old rat, hepatic PEPCK activity was elevated by HEOD at three hours. Furthermore, adult rats exhibit enhanced gluconeogenic enzyme activity after HEOD and a prolonged fast.

The characteristic hyperglycemic response induced in the adult rat by chlorinated hydrocarbon insecticides results from both enhanced gluconeogenesis and glycogenolysis (Bhatia et al., 1972; Kacew & Singhal, 1973a, 1973b; Kacew et al., 1972a). If the HEOD-induced carbohydrate response in the young rat is maintained initially through enhanced gluconeogenesis then a lack of gluconeogenic substrates would effectively and rapidly bring about a conclusion of the response.

The suckling rat is remarkably insensitive to the level of circulating blood glucose and makes little attempt to regulate it even in conditions which produce hypoglycemia (Gil-Ad et al., 1975; Hetenyi & Cowan, 1980). This decreased sensitivity to glucose is consistent with the minor nutritional role of the substrate in the immature rat. In fact, glucose utilization in the one to twelve day old rat is only one-tenth that seen in the adult (Moore et al., 1971). On the other hand, the suckling rat is sensitive to alterations in the circulating

levels of ketone bodies (Stumpf & Kraus, 1979) and closely regulates these metabolites (Hahn, 1970). Fasting in the young rat effectively ceases the supply of dietary lipids, the major source of ketones, and therefore the animal would be expected to activate mechanisms which ensure the required supply of ketones is not decreased. This dependancy on lipid rather than carbohydrate may explain why the HEOD-induced carbohydrate response is not maintained in the immature rat.

Dieldrin briefly enhanced glycogenolysis in the young rat. The catabolism of hepatic glycogen provides a pool of glucose-6-phosphate which in the presence of high G6P activity is readily converted into glucose (Mayes, 1977b). However, in the immature HEOD-treated rats, hepatic G6P activity was not increased over that found in the non-HEOD treated animals. Thus, despite an increased rate of glycogenolysis, the rate of gluconeogenesis derived from glucose-6-phosphate might not have been elevated in the HEOD-treated pups. If the surplus glucose-6-phosphate produced in these animals was recycled back into glycogen (Mayes, 1977b) then one might not detect any significant change in the level of hepatic glycogen.

However, it is also possible that some surplus glucose-6-phosphate may enter the hexose-monophosphate shunt (Mayes, 1977b). In the adult rat, HEOD has been shown to increase the activity of several of the enzymes of the hexose-monophosphate shunt pathway (Kohli et al., 1975b). If this occurred the glucose-6-phosphate could ultimately be converted into fructose-6-phosphate and glyceraldehyde-3-phosphate (Mayes,

1977b). These products could in turn be used in the glycolytic pathway to produce phosphoenolpyruvate (Mayes, 1977b). In order for fructose-6-phosphate to be so utilized, hepatic FDP activity would have to be low, a condition which was seen in the 10 day old pup at three hours after HEOD treatment. In order for glyceraldehyde-3-phosphate to be converted into phosphoenolpyruvate, gluconeogenesis from glycerol would have to be reduced, a condition known to develop rapidly in the fasting young dog (Hall et al., 1976).

Dieldrin increased hepatic PEPCK activity at three hours in the young rat, yet no hyperglycemia developed in consequence. PEPCK catalyses the conversion of oxaloacetic acid to phosphoenolpyruvate (Mayes, 1977b). Phosphoenolpyruvate may be utilized in the gluconeogenic pathway, to produce glyceraldehyde-3-phosphate, or via the glycolytic pathway, to produce pyruvate (Mayes, 1977b). Provided glyceraldehyde-3-phosphate levels are high, which may be the case in the immature HEOD-treated rat, pyruvate production would be favored. Increased pyruvate should cause increased production of acetyl CoA. Acetyl CoA levels are usually increased as a consequence of the oxidation of dietary fatty acids in the fed suckling rat (Hahn, 1970). However, in the fasting rat there should exist an increased demand for acetyl CoA to combat the loss of dietary fatty acids.

Therefore, in the immature, fasted HEOD-exposed rat a HEOD-induced increase in hepatic PEPCK activity may actually lead to an increased production of ketone bodies, rather than

to enhanced gluconeogenesis as in the adult (see figure 13). Thus, it is possible that as a result of the lipid requirements of the fasted young rat, the usual HEOD-induced carbohydrate response may be altered.

D. Toxic versus adaptive nature of HEOD-induced hyperglycemic response in the adult rat.

The question as to whether the hyperglycemic response induced by organochlorine insecticides is protective to the exposed animal has not been adequately addressed. Only Stohlman & Lillie (1948) have investigated the effects of altered carbohydrate metabolism on the toxicity of one of these agents. These authors concluded that in the adult rabbit treated chronically with both DDT (150 mg/kg/day p.o. for 10 days) and d-glucose (1-5 g/kg/day i.v. for 10 days), glucose decreased the frequency of DDT-induced pathologic lesions in some organs. However, glucose did not decrease the overall mortality caused by the pesticide. Thus a major goal of this current study was to determine the protective value of the hyperglycemic response.

There was reason to believe that the response might enhance the acute toxicity of dieldrin. For example, in the adult mammal HEOD produces both hyperglycemia (Hiddemen & Cornish, 1970; Bhatia et al., 1973; Kohli et al., 1975b) and CNS hyperactivity (Gowdy et al., 1954; Gowdy & Stavaky, 1955). The CNS hyperactivity is the ultimate cause of death, since the increased vagal stimulation eventually causes cardiac arrest (Gowdy et al., 1954).

Figure 13. Possible pathways utilized in carbohydrate metabolism in the fasted, HEOD-treated immature rat.

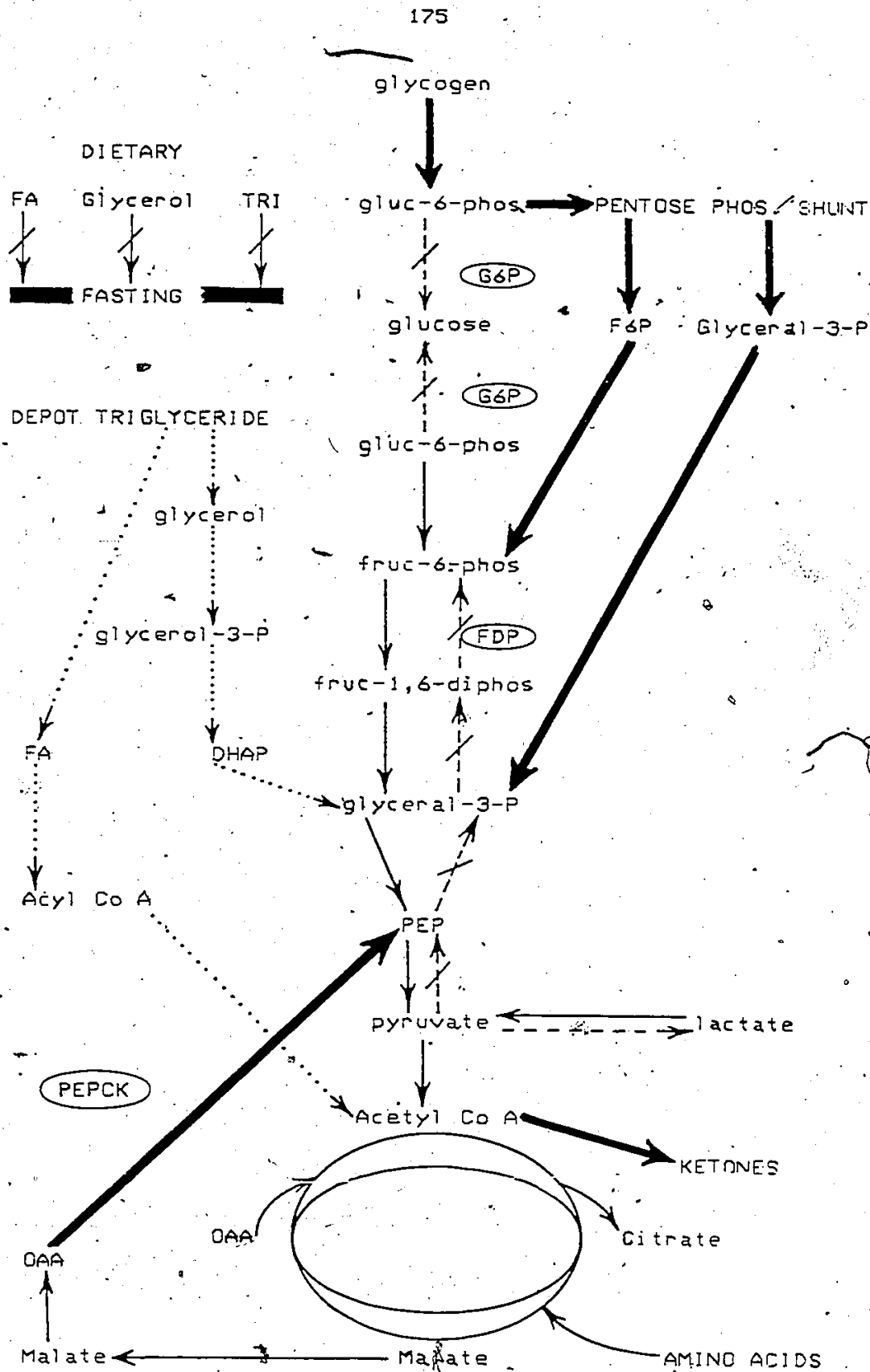
DHAP: dihydroxyacetone phosphate
 FA: fatty acid
 F6P: fructose-6-phosphate
 fruc-6-phos: fructose-6-phosphate
 fruc-1,6-phos: fructose-1,6-diphosphate
 gluc-6-phos: glucose-6-phosphate
 glyceral-3-P: glyceraldehyde-3-phosphate
 glycerol-3-P: glycerol-3-phosphate
 OAA: oxaloacetic acid
 PEP: phosphoenolpyruvate
 TRI: triglyceride

(FDP): fructose-1,6-diphosphatase

(G6P): glucose-6-phosphatase

(PEPCK): phosphoenolpyruvate carboxykinase

—: major operative pathway (carbohydrate)
 - - - : blocked/spared pathway (carbohydrate)
 ←: HEOD stimulated pathway (carbohydrate)
 ←.....: major operative pathway (lipid metabolism)



The non-ketogenic adapted mammal exhibits an absolute requirement for glucose in order to maintain CNS function (Ensinck & Williams, 1981). In the HEOD-treated animal, the increased activity of the CNS might increase its demand for glucose. Cerebral cells absorb glucose directly from the blood through facilitated diffusion. Therefore, it is possible that any treatment which acts to decrease the blood glucose concentration in the HEOD-exposed animal may also reduce the acute toxicity of dieldrin. Conversely, any treatment which increases the hyperglycemia produced in these animals might also enhance the toxicity of HEOD.

Elevation of the hyperglycemia with d-glucose did not increase the acute mortality of dieldrin. Thus, increasing the hyperglycemia confers no adaptive advantage to the HEOD-exposed rat. This is essentially the same result found for glucose treatment in the adult rabbit chronically exposed to DDT by Stohlman & Lillie (1948).

However, it is also possible that a hyperglycemia greater than that normally caused by HEOD alone may be superfluous to the maintenance of the hyperactive CNS. This might explain the failure of potentiated hyperglycemia to enhance the acute toxicity of dieldrin.

There is evidence that the normal hyperglycemia seen in the HEOD-exposed rat may enhance the toxicity of the compound. Treatments (i.e. phenobarbital, atropine and l-a-methyldopa) which prevent or attenuate the HEOD-induced hyperglycemic response also reduce the mortality caused by the pesticide.

However, these treatments did not also always cause a reduction in HEOD-induced hyperstimulation of the CNS.

Recall that phenobarbital eliminated both the convulsions and the mortality, yet only attenuated the hyperglycemia caused by dieldrin. Phenobarbital and glucose treatment restored both the hyperglycemia and the 24 hour mortality to levels typical in rats given only HEOD. Yet during the early period of this latter experiment, when hyperglycemia was restored but the convulsions were inhibited, no mortality occurred. Rather, it was only after HEOD-induced hyperactivity of the CNS had been initiated in these animals that the mortality began to occur. Thus the hyperglycemia, per se, is not actually toxic.

On the other hand, in the atropine experiment, HEOD-induced CNS hyperstimulation was not associated with mortality until hyperglycemia had become established. Similarly, 1- α -methyldopa did not alter HEOD-induced CNS hyperactivity but did inhibit briefly, the HEOD-induced hyperglycemic response. During the time in which the hyperglycemia was inhibited no mortality occurred. Thus, these data suggest that the HEOD-induced hyperglycemic response is a necessary component of the acute toxicity of the insecticide.

Conversely, mortality and CNS hyperactivity were not changed in the adult rat given HEOD and 2-deoxyglucose. The enhanced hyperglycemia seen in these animals was considered indicative of the establishment of CNS intracellular glucose deficiency. Thus there is some reason to believe that the

CNS-elicited effects of dieldrin may occur independantly of the CNS intracellular glucose concentration. Unfortunately since the CNS intracellular glucose concentration in HEOD and 2-deoxyglucose treated rats was not actually measured, it is difficult to objectively assess the true importance of the conclusion presented above. However, some support for the above view is forthcoming from experiments involving insulin-treatment in the HEOD-exposed rat.

The insulin experiments clearly indicated that potentiation of HEOD-induced CNS hyperactivity will increase the toxicity of the compound even in the complete absence of hyperglycemia. These experiments also indicate that hyperglycemia is not required to maintain the CNS hyperactivity and therefore suggest that the HEOD-induced hyperglycemic response may be inconsequential to the acute toxicity of dieldrin.

However, these insulin experiments are also somewhat difficult to interpret, since insulin by inducing hypoglycemia will initially cause CNS hyperactivity itself (Ensinck & Williams, 1981). Recall that the latency to convulsions in the HEOD and insulin-treated rat was only approximately one-half that seen in the rat given HEOD alone.

Thus, we are unable to ascertain the significance of the hyperglycemic response as it relates to the toxicity of HEOD. However, our results do suggest a possible experimental design which might provide a definitive answer to this question.

Ideally such an experiment would block the HEOD-induced hyperglycemia but still maintain plasma glucose at euglycemic.

levels and not interfere with or otherwise alter the HEOD-induced CNS hyperstimulation. If under these conditions, mortality was not altered, then one could conclude that the hyperglycemic response plays no role in the acute toxicity of dieldrin.

However, without first achieving a better understanding of the fundamental mechanism by which the HEOD-induced carbohydrate response is elicited, such an idealized experiment as that described above can probably not be performed. Therefore, it was decided to focus further attention on the etiology of the response.

E. Etiology of the HEOD-induced carbohydrate response in the adult rat.

Ei. The literature

The etiology of the organochlorine-induced carbohydrate response is unknown. However, several explanations have been proposed. Stohlman & Lillie (1948) suggested that the response might result as a consequence of organochlorine-induced adrenocortical hyperactivity. Alternatively, these authors suggested that the hyperglycemia represented a homeostatic response to ensure that increased glucose demand of muscles would be met. The increased demand for glucose in muscle was thought to occur as an indirect result of organochlorine-induced CNS hyperactivity.

Hiddemen & Cornish (1970) and Bhatia et al. (1971, 1972) have all attributed the hyperglycemic response to the effects of enhanced adrenal cortical activity. However, Kacew et al.

(1972a), Kacew & Singhal (1973a) and Hickenbottom & Yau (1974) have demonstrated that the hyperglycemic response is inducible in the adrenalectomized rat. Neither, does the glucocorticoid triamcinolone potentiate the response in the adrenalectomized rat (Kacew & Singhal, 1973a). Thus the response is neither initiated nor influenced to any significant extent by adrenal-corticoid activity.

Kacew & Singhal (1973b, 1973c, 1974a, 1974b) found that DDT and other organochlorine pesticides increased the intracellular cAMP concentration in rat renal cortex, enhanced renal and hepatic cyclase activity and inhibited the activity of renal phosphodiesterase. Based on these results, Kacew & Singhal (1974a, 1974b) have proposed a model to explain the organochlorine-induced carbohydrate response which centres on increased cAMP. These authors believe that chlorinated hydrocarbon pesticides can enhance the activity of renal and hepatic adenylyl cyclase and thus increase the intracellular concentration of cAMP. These authors' results provoke speculation that the organochlorines "mimic" the effects of glucagon and so elicit the hyperglycemic response.

Kacew & Singhal's observation of increased adenylyl cyclase activity following exposure to chlorinated hydrocarbon agents has not been confirmed by other workers. Poschl et al. (1982) found the basal activity of adenylyl cyclase was not changed in hepatocytes obtained from rats acutely or chronically exposed to DDT. Dudeja et al. (1980) reported that in the DDT-treated rhesus monkey, hepatic adenylyl cyclase

activity is reduced when compared to that seen in non-DDT exposed controls. Thus the ability of the organochlorine pesticides to increase adenylyl cyclase activity is controversial.

Also, there is some doubt as to the physiological significance of an organochlorine caused increase in adenylyl cyclase activity. Story & Freedland (1978a, 1979) found that feeding DDT to rats actually decreased the ability of cAMP to stimulate gluconeogenesis (from lactate) in isolated hepatocytes.

Little work has been performed investigating the effects of chlorinated hydrocarbon pesticides on circulating levels of insulin or glucagon. Yau & Mennear (1977) found plasma immunoreactive insulin (IRI) was unchanged in mice, 18 hours after administration of a small non-hyperglycemia provoking dose of DDT. However, the glucose tolerance and the release of IRI from the pancreatic islets were decreased. These authors concluded therefore, that DDT could inhibit the pancreatic release of insulin in the face of stimulatory challenge. If this conclusion is correct, it might explain the hyperglycemia known to follow higher doses of organochlorine pesticides.

Plasma glucagon levels have not been measured in the animal exposed to organochlorine agents. However, Story & Freedland (1978a, 1979) have found glucagon-stimulated gluconeogenesis (from lactate) was reduced in hepatocytes obtained from rats fed DDT. These authors have suggested that DDT may cause a decreased sensitivity to glucagon in rat liver. How-

ever, this effect can be overcome in vitro by high concentrations of the hormone (Story & Freedland, 1979). Finally, Poschl et al. (1982) have reported that in vitro glucagon-stimulated adenylyl cyclase activity is reduced when DDT is added to rat hepatocyte incubates. These studies suggest that exposure to organochlorine pesticides may reduce the release of insulin while simultaneously decreasing the sensitivity of the hepatocyte to glucagon.

Eii. A new model to explain the elicitation and control of the HEOD-induced hyperglycemic response.

The results of this study suggest a model for the etiology of the HEOD-induced hyperglycemic response. This model proposes that HEOD induces an increased release of pancreatic glucagon and a simultaneous decrease in the release of insulin. However, prior to describing the model, a brief review of the neurological control of the endocrine pancreas in the normal animal will be presented.

The CNS centres for the neurological control of the mammalian endocrine pancreas are located in the ventral hypothalamus (Porte & Halter, 1982). Two distinct sub-centres are located in this region, the ventomedial loci (VMH) and the ventrolateral nucleus (VLH). The VMH is thought to represent a sympathetic centre, and the VLH a parasympathetic centre (Woods & Porte, 1974). Neural stimuli arising in the VMH exit the hypothalamus via the splanchnic nerves, while stimuli from the VLH are conducted by the vagi (Smith & Porte, 1976; Porte & Halter, 1982). The splanchnic and vagus nerves

combine to form the mixed pancreatic nerve which innervates the endocrine pancreas (Woods & Porte, 1974). Thus the endocrine pancreas of all mammals (except the "Spiny" mouse Acomys cahirinus) is innervated by both the sympathetic and the parasympathetic systems (Smith & Porte, 1976).

There are species differences in the relative proportions of adrenergic and cholinergic efferents present in the pancreas (Woods & Porte, 1974). For example, the rat pancreas receives many cholinergic fibers (Morgan & Lobl, 1966) but relatively few adrenergic fibers (Cegrell, 1968).

However, these anatomical differences in nerve supply may have little physiological significance, as according to Smith & Porte (1976), all nerve fibers which enter pancreatic islets end blindly at a distance of 20-30 μ m from the secretory cells. These authors believe that neurotransmitters are released into the intercellular space and consequently stimulate or inhibit many secretory cells simultaneously. There also exists a large number of tight and gap-junctions between islet cells of all types (Unger et al., 1978). These intercellular junctions may serve to further disseminate neural input.

Marliss et al. (1972, 1973) have shown, in the dog, that in vivo electrical stimulation of the pancreatic nerve causes a prompt increase in pancreatic release of glucagon. Glucose infusion will inhibit this effect. However, the effect is not inhibited by the administration of atropine, indicating that it is primarily of sympathetic origin. Stimulation of

the pancreatic nerve in the isolated dog pancreas briefly enhances the release of insulin (Porte et al. 1973). However, this effect persisted for only five minutes despite continued stimulation of the pancreatic nerve. This effect is also readily blocked by atropine, indicating parasympathetic control.

Studies such as those cited above involving electrical stimulation of the mixed pancreatic nerve do not clearly differentiate between sympathetic and parasympathetic effects unless appropriate blocking agents are used. For this reason most authors choose to study the effects of stimulating or ablating discrete sympathetic or parasympathetic pathways leading to the endocrine pancreas.

Hence, the bilateral destruction of the VMH in the rat results in chronically increased plasma insulin levels (Woods & Porte, 1974; Smith & Porte, 1976) and hypertrophy and hyperplasia of pancreatic β -cells (Woods & Porte, 1976; Gerich et al., 1976). Conversely, electrical stimulation of the VMH in the rat decreases plasma insulin level and increases both plasma glucagon and glucose levels (Frohman & Bernardis, 1971; Gerich et al., 1976). According to Gerich et al. (1976) adrenalectomy can reverse the decrease in plasma insulin but it does not inhibit either the increase in plasma glucose or glucagon. Therefore the glucagon effect results as a consequence of direct sympathetic innervation, while the insulin effect may be due to an enhanced release of adrenal catecholamines.

Electrical stimulation of the splanchnic nerves in the cat

(Esterhuizen & Howell, 1970) and the calf (Bloom et al., 1973) increases the release of pancreatic glucagon and also causes hyperglycemia. Stimulation of this nerve in the dog increases the secretion of glucagon and decreases the release of insulin (Porte et al., 1973; Gerich et al., 1976).

Stressful stimuli which enhance sympathetic activity, such as exsanguination, myocardial infarct or burn trauma will also cause increased glucagon secretion while simultaneously inhibiting the release of insulin (Unger et al., 1978). Lindsey et al. (1975) showed that enhanced glucagon release can still be induced by cardiovascular shock even in the presence of hyperglycemia. These authors concluded that this glucagon effect was mediated by β -adrenergic receptors, since it was abolished by propranolol treatment.

However, the increased release of glucagon in response to stress may not be solely mediated by the sympathetic nervous system. In the sheep, glucagon release induced by exsanguination can be prevented with atropine (Haimagyi et al., 1969). Also, Vaughan et al. (1973) found that sectioning the splanchnic nerves in calves did not prevent the glucagon release caused by insulin-induced hypoglycemia. However, when atropine was administered to these asplanchnic animals, pancreatic glucagon secretion was significantly decreased in the face of hypoglycemia. Thus, it seems probable that both the sympathetic and the parasympathetic systems are involved in the mediation of the glucagon-stress response.

It has been shown, both *in vivo* and *in vitro*, that catech-

cholamines act on the pancreatic islet to increase glucagon secretion and inhibit insulin release (Robertson & Porte, 1973a, 1973b; Woods & Porte, 1974; Gerich et al., 1976; Smith & Porte, 1976).

In summary, it is now generally accepted that the primary effects of sympathetic stimulation on the mammalian endocrine pancreas is an inhibition of insulin release mediated by α -adrenergic receptors coupled with a stimulation of glucagon release mediated by β -adrenergic receptors (Woods & Porte, 1974; Gerich et al., 1976; Smith & Porte, 1976; Unger et al., 1978; Samols & Weir, 1979; Porte & Halter, 1982).

Turning our attention to the effects of parasympathetic stimulation, bilateral destruction of the VLH in mice (Woods & Porte, 1974) and in rats (Steffens et al., 1972; Gerich et al., 1976) decreases plasma insulin levels. Conversely, electrical stimulation of this nucleus increases insulin secretion in the rat (Steffens et al., 1972; Woods & Porte, 1974; Gerich et al., 1976).

Vagotomy in the rat does not alter either basal pancreatic insulin release or blood glucose concentration (Woods & Porte, 1976). However, section of the vagi does abolish the ability of the rat to release insulin in response to conditioned stimuli (e.g. olfactory, gustatory or visual) which would otherwise cause an enhanced release of this hormone (Gerich et al., 1976; Smith & Porte, 1976). In man, vagotomy impairs the release of both pancreatic insulin and glucagon (Smith & Porte, 1976) and decreases glucose tolerance (Woods & Porte, 1974).

Vagotomy also reduces the circulating insulin level in the fasted monkey (Woods & Porte, 1974). Finally, in the dog, section of the vagi decreases insulin secretion for a period of one hour, but this effect does not alter blood glucose level (Frohman et al., 1967).

Electrical stimulation of the vagus is generally believed to cause the release of insulin (Woods & Porte, 1974; Gerich et al., 1976; Smith & Porte, 1976) and glucagon (Gerich et al., 1976; Smith & Porte, 1976). The increase in glucagon secretion has been accompanied by elevated blood glucose levels in both the dog (Kaneto et al., 1974) and the calf (Bloom et al., 1974). An increase in insulin secretion from vagal stimulation will result only if blood glucose levels are high (circa 150 mg/dl - Smith & Porte, 1976; Halter & Porte, 1982).

Additionally, there is considerable evidence that this enhanced insulin secretion is not only extremely short-lived but is also of little importance to the control of blood glucose. For example, in the dog, vagal stimulation causes an immediate and large increase (as much as 150%) in the basal secretion of insulin (Frohman et al., 1967; Kaneto et al., 1967, 1974). However, this effect is transient, persisting for at most six minutes, and is followed by a return to normal secretory levels even if vagal stimulation is continued (Frohman et al., 1967; Kaneto et al., 1967, 1974).

In view of the short duration of this insulin effect, it is perhaps not surprising that the above authors could detect no decrease in circulating blood glucose level after vagal

stimulation. In fact, Frohman et al. (1967) found the blood glucose concentrations in their animals were actually increased after vagal stimulation. Daniel & Henderson (1967) found similar results upon vagal stimulation in the baboon. These investigators showed that 10 minutes of vagal stimulation could increase pancreatic insulin secretion by 30%. However, despite the enhanced insulin release, blood glucose level did not change. Vagal stimulation of the isolated dog pancreas increases the release of insulin by 30% (Bergman & Miller, 1973). This enhanced insulin output persists for only three to four minutes, after which time, insulin secretion returns to normal levels despite continued vagal stimulation.

These authors have suggested that vagal stimulation can only cause the release of the small labile pool of insulin from the pancreas. In their view, vagal stimulation does not cause long-term insulin release and is not important in the long-term regulation of pancreatic β -cell function. This would explain the transient nature of the vagally mediated insulin response, and also explain why blood glucose levels are not decreased following vagal stimulation.

When vagal stimulation is combined with atropine treatment in the dog, the drug not only blocks the vagally mediated increase in insulin release (Frohman et al., 1967; Kaneto et al., 1974) but also will decrease the basal level of pancreatic glucagon release (Kaneto et al., 1974). Diminished glucagon output after atropine treatment has also been reported for man (Gerich et al., 1976) rats (Smith & Porte, 1976) and

the calf (Vaughan et al., 1973; Bloom et al., 1974).

The in vivo administration of parasympathomimetic drugs causes increased release of both insulin and glucagon in dogs, mice and men (Woods & Porte, 1974; Gerich et al., 1976; Smith & Porte, 1976; Unger et al., 1978). These drugs cause identical effects in vitro. For example, Loubatieres et al. (1970) found that in isolated rat pancreas, acetylcholine caused the release of the labile pool of insulin. Malaisse et al. (1967) demonstrated that this effect in rat pancreas would only occur provided the glucose concentration in the incubate medium was at least 100 mg/dl. These authors also showed that acetylcholine and carbonyl chloride were ineffective in increasing insulin in incubates which had been treated with atropine.

Parasympathomimetics also stimulate pancreatic glucagon release in vitro (Woods & Porte, 1974; Gerich et al., 1976; Unger et al., 1978). In fact, acetylcholine can stimulate the release of glucagon in isolated canine pancreas even in the face of normally inhibitory concentrations of glucose (Iversen & Arhus, 1973). Atropine is effective both in vivo (Bloom et al., 1974; Gerich et al., 1976) and in vitro (Iversen & Arhus, 1973; Gerich et al., 1976; Smith & Porte, 1976) in blocking parasympathomimetic stimulation of pancreatic glucagon release. Thus the current view is that parasympathetic stimulation causes an increase in both pancreatic insulin and glucagon release (Gerich et al., 1976; Smith & Porte, 1976; Unger et al., 1978; Porte & Halter, 1982). However, the

increase in insulin secretion is both transient and relatively unimportant in blood glucose regulation (Frohman et al., 1967; Kaneto et al., 1967, 1974; Daniel & Henderson, 1967; Loubatieres et al., 1970; Bergman & Miller, 1973). Therefore, the major consequence of increased parasympathetic stimulation of the pancreas is an increase in the release of glucagon.

Eiii. The model.

A diagram of the proposed model is presented in Figure 14. Diethylmercaptol (DEM) is a known CNS stimulant (Gowdy et al., 1954; Gowdy & Stavsky, 1955) and therefore probably causes stimulation of both the VMH and the VLH nuclei. Stimulation of these nuclei would increase neural output in both the vagal and splanchnic nerves. Diethylmercaptol is known to increase vagal activity to the heart (Gowdy et al., 1954). Activity in the splanchnic nerves of the insecticide-exposed animal has not been measured. However, since these compounds elicit convulsions, generalized sympathetic activity is probably increased. It seems reasonable that enhanced neural activity in the VMH-splanchnic and VLH-vagal pathways would be accompanied by enhanced activity in the pancreatic nerve. Consequently, the model proposes that because DEM induces CNS hyperstimulation, both sympathetic and parasympathetic stimulation of the pancreas is increased.

An increase in sympathetic stimulation of the pancreas of the DEM-treated animal should elicit two effects. First, occupancy of α and β receptors on islet cells would be increased. α -adrenergic stimulation of the β -cell is known

Figure 14. Diagrammatic representation of the model proposed to explain the etiology of the HEOD-induced hyperglycemic response in the adult rat.

CNS: central nervous system (thalamus)

(VMH): ventromedial nuclei (hypothalamus)

(VLH): ventrolateral nuclei (hypothalamus)

a: alpha-adrenergic receptor

b: beta-adrenergic receptor

ACH: cholinergic receptor (muscarinic)

■ or ■ : drug-induced blockade of receptor

AT: atropine

L-a-MD: L-a-methyldopa

PB: phenobarbital

PR: propranolol

FA: fatty acid

GLUCONEO.: gluconeogenesis

GLYCOGEN.: glycogenolysis

DEC.: decrease level

INC.: increase level

NOR.: normal level

← : primary effect produced is stimulation

— : primary effect produced is inhibition

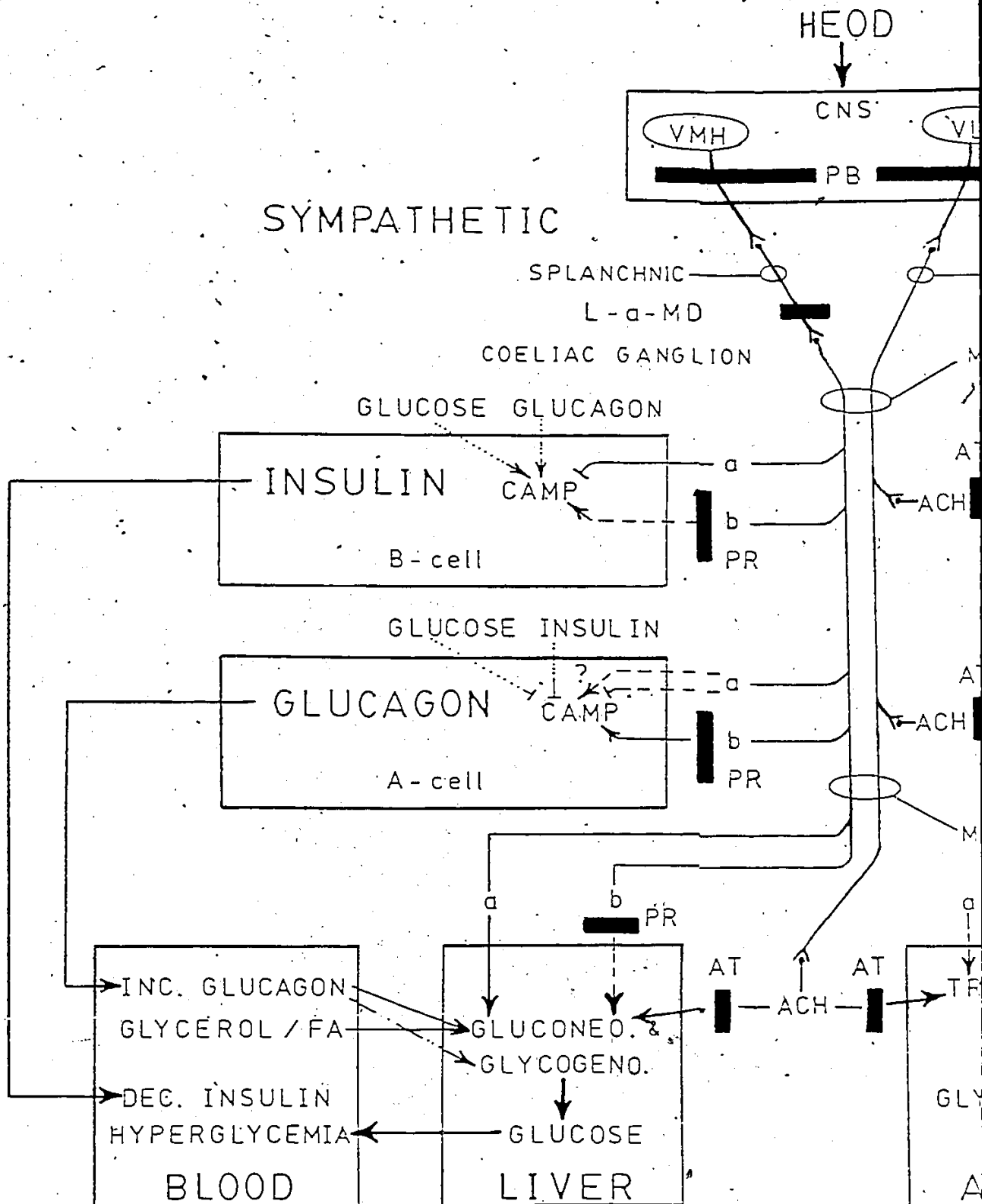
← - - - : secondary effect produced is stimulation

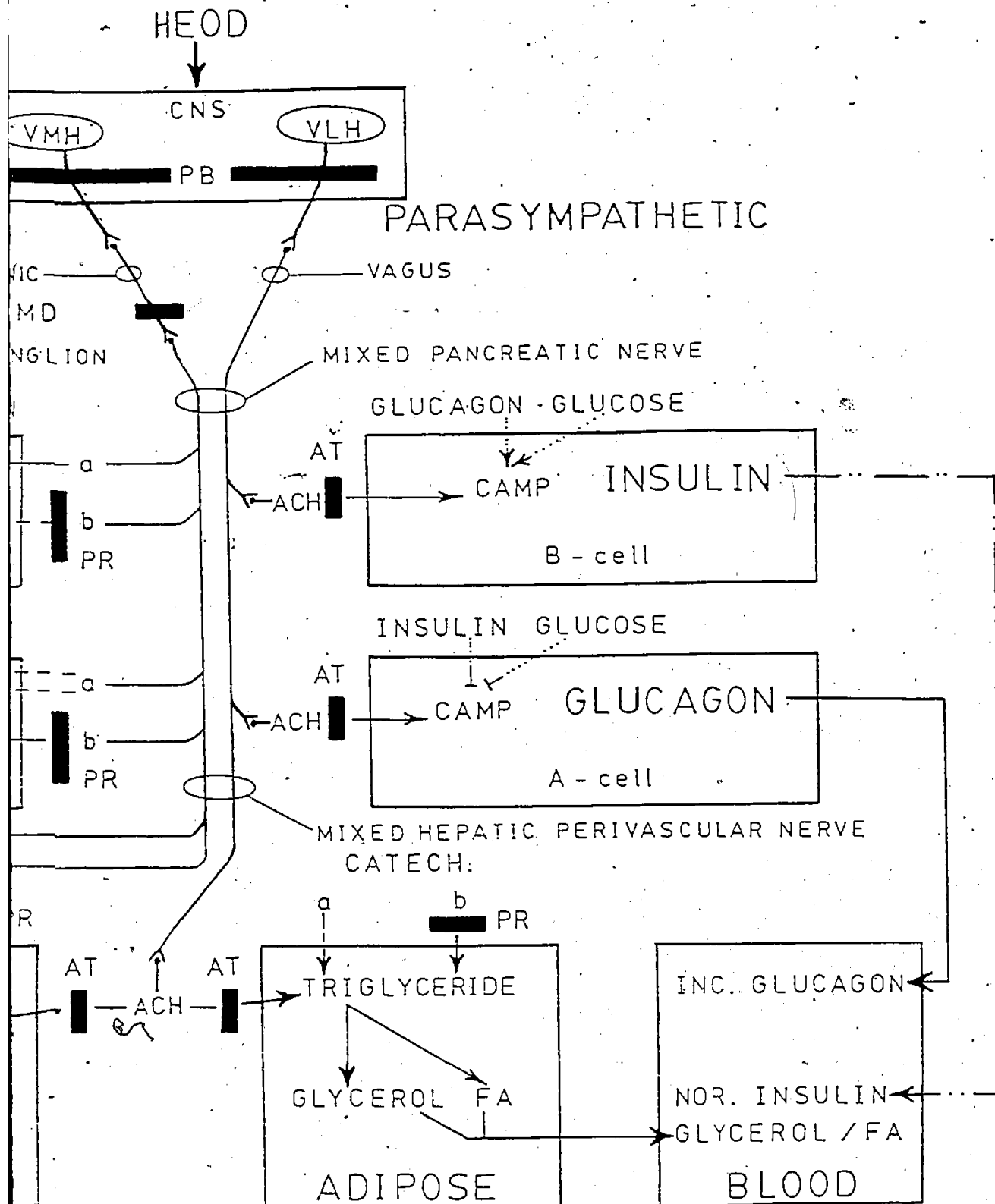
— - - - : secondary effect produced is inhibition

← ··· : transitory effect

← ····· : non-neuronal effect (stimulation)

— ····· : non-neuronal effect (inhibition)





to inhibit the release of insulin; conversely, β -adrenergic stimulation has the opposite effect (Gerich et al., 1976; Smith & Porte, 1976; Unger et al., 1978; Halter & Porte, 1982).

However, in the pancreas of the normal animal, α -receptor effects predominate (Smith & Porte, 1976). Therefore, we propose that in the HEOD-treated rat, insulin secretion is inhibited.

β -adrenergic stimulation of the α -cell causes an increase in the release of glucagon (Gerich et al., 1976; Smith & Porte, 1976; Porte & Halter, 1982). However, α -adrenergic effects in the α -cell are still controversial. There is evidence that stimulation of the α -receptor may either inhibit and/or stimulate glucagon release (Woods & Porte, 1974; Gerich et al., 1976). However it has been well established that in the α -cell, β -receptor mediated effects predominate. Thus we propose that in the pesticide-exposed rat, glucagon secretion would increase as a consequence of β -adrenergic activity.

As reviewed above, parasympathetic stimulation of the pancreas of the normal animal also has two effects. First, cholinergic stimulation of the β -cell causes a transient increase in the release of insulin, provided blood glucose levels are high (Porte & Halter, 1982). However, this brief surge of insulin is unimportant in the regulation of plasma glucose concentrations (Frohman et al., 1967; Daniel & Henderson, 1967; Kaneto et al., 1967, 1974). Thus, in the HEOD-treated rat, increased vagal activity may cause a brief elevation in insulin secretion, but it would not significantly

alter the plasma glucose concentration. In fact, since α -adrenergic stimulation would inhibit insulin release, increased vagal stimulation might not actually enhance insulin secretion.

We propose therefore that the major effect produced in the pancreas of the HEOD-treated rat by increased cholinergic stimulation is an enhanced secretion of glucagon. In the normal animal, this enhanced glucagon release is not inhibited by elevated plasma glucose levels (Iversen & Arhus, 1973). Furthermore, since β -adrenergic stimulation also increases pancreatic glucagon release, it is probable that the paramount effect produced by HEOD-induced CNS hyperstimulation in the rat is a greatly enhanced pancreatic glucagon release.

In the normal animal, glucagon is known to stimulate hepatic glycogenolysis, gluconeogenesis and ketogenesis (Ensinck & Williams, 1982). According to these authors, "It has recently been shown that the effects on glycogenolysis are evanescent, with a "down-regulation" of the glucagon receptor, leading to a drop in enzyme activities involved with glycogenolysis. In contrast, gluconeogenesis stimulated by glucagon persists for long periods in the post-absorptive state." The primary enzyme which regulates glucagon-induced gluconeogenesis is believed to be pyruvate carboxylase (Grodsky, 1977). In adipose tissue, glucagon stimulates lipolysis, and the fatty acids and glycerol, so produced are used to promote ketogenesis and gluconeogenesis in the liver (Grodsky, 1977; Ensinck & Williams, 1982).

Thus in the HEOD exposed rat, the characteristic hyperglycemic response is proposed to result as a direct consequence of enhanced pancreatic glucagon release, perhaps coupled with diminished release of insulin. These pancreatic effects are in turn believed to result as a consequence of increased parasympathetic and sympathetic activity arising from HEOD-induced CNS hyperstimulation.

Eiv. Experimental support for the model.

All of the data of this study are compatible with the proposed model.

The barbiturates cause a widespread depression in the mammalian CNS (Sharpless, 1970). Thus, the motor cortex, thalamus, hypothalamus, limbic system, reticular formation and spinal cord all exhibit decreased spontaneous electrical activity following barbiturate exposure (Domino, 1962). The hypothalamus is especially sensitive to the depressant effects of these drugs, even more so than is the midbrain reticular formation (Domino, 1962). Feldman et al. (1959) extensively studied the effects of pentobarbital on evoked potentials in various hypothalamic nuclei in vivo, in cats. These authors found that as little as 10 mg/kg (i.v.) of pentobarbital significantly depressed all areas of the hypothalamus and that doses of 15 - 20 mg/kg (i.v.) would completely abolish these potentials. Conversely, anesthetic doses, (35 - 40 mg/kg i.v.) were required to abolish the potentials in the reticular formation.

Administration of phenobarbital to the HEOD-treated rat attenuated hyperglycemia and virtually abolished convulsions.

Phenobarbital should block or at least greatly attenuate HEOD-induced hyperactivity in both the VLH and VMH nuclei. Thus according to the model, glucagon release would not be stimulated. The administration of α -adrenergic blocking agents such as phentolamine reverses α -adrenergic induced inhibition of pancreatic insulin release in rats, mice, dogs and baboons (Woods & Porte, 1974; Smith & Porte, 1976). These results have led Woods & Porte (1974) to suggest that in the absence of α -adrenergic stimulation, pancreatic insulin secretion is increased. Thus phenobarbital treatment, might act to reduce plasma glucose concentrations to below normal levels.

This in fact was found in control rats treated with oil and phenobarbital. Plasma glucose concentrations in these animals were reduced by 25%. Similarly, the highest plasma glucose level in the rat treated with HEOD and phenobarbital was 34% lower than that in rats given HEOD alone. Thus, phenobarbital was equally effective in lowering plasma glucose in both the control and the treated rats of this experiment.

Bailey et al. (1979) reported that chronic exposure of adult mice to pentobarbital (45 mg/kg/day i.v. for 10 days) resulted in a 50% decrease in plasma insulin concentration and a 20% increase in fasting plasma glucose concentration. Bailey & Flatt (1980) also found that a single dose of pentobarbital (45 mg/kg i.p.) in the mouse, increased plasma glucagon level by about 15% and caused a mild transient hyperglycemia (maximal increase over control blood glucose level, 28%). Both these effects occurred early after the

development of anesthesia (i.e. in the first 5 to 10 minutes). Plasma insulin concentrations in the barbiturate-treated mice were maximally increased by 80%. However this insulin effect developed gradually to reach a peak level after 90 minutes of anesthesia had passed.

The results of these two studies do not necessarily contradict those expected following barbiturate exposure in our model. The above studies utilized a different species, a different barbiturate and most importantly, involved no exposure to a pesticide. Therefore, it is not surprising that the results found in regard to plasma glucose were also different from those determined by us. Regardless, our experimental data clearly indicate that phenobarbital attenuates HEOD-induced hyperglycemia in the rat.

Exogenous insulin caused an equivalent degree of hypoglycemia (as reflected by absolute plasma glucose levels) in both the non-HEOD and the HEOD-exposed rat. Therefore, HEOD does not alter the physiological consequence of a pharmacological dose of insulin. Consequently, endogenous insulin may also be equally potent in either the non-HEOD or the HEOD-exposed rat.

Exogenous glucose increased hyperglycemia to a greater extent in the HEOD-treated rat than it did in the non-HEOD treated animal. Elevated plasma glucose in the normal animal stimulates the release of insulin and inhibits the release of glucagon (Unger et al., 1978; Porte & Halter, 1982). Thus, plasma glucose homeostasis is restored. According to the model,

these homeostatic mechanisms do not operate normally in the HEOD-exposed rat. Thus, the increased hyperglycemia found in the HEOD and d-glucose-treated rat is both predicted and explained by the model.

L-a-methyldopa blocks sympathetic transmission in the CNS (Henning, 1969; Weiner, 1980) and acts peripherally to decrease sympathetic transmission by causing the synthesis of a false neurotransmitter which is subsequently released in place of norepinephrine (Weiner, 1980). In the proposed model, L-a-methyldopa treatment in the HEOD-exposed rat would block neural transmission through the splanchnic nerves. Provided that parasympathetic activity was still elevated, then the loss of the glucagon released by β -adrenergic stimulation might be of little consequence to the HEOD-induced hyperglycemic response, since cholinergic mediated glucagon release could still occur.

In the pancreatic β -cell, on the other hand, the loss of sympathetic stimulation will abolish the α -adrenergic inhibition of insulin release. Therefore, with L-a-methyldopa treatment, one would expect a decrease in the hyperglycemia induced by diethrin. This was observed, as l-a-methyldopa reduced HEOD-induced hyperglycemia by 25%. This relatively moderate diminution also indicates that the majority of the hyperglycemia induced by HEOD is caused by cholinergic stimulation, confirming the results seen with atropine.

Propranolol specifically blocks β -adrenergic receptors (Nickerson, 1970). With a β -adrenergic block on the pancreatic islet cells, enhanced sympathetic stimulation would not

induce either glucagon or insulin release. However, enhanced sympathetic activity would still be able to elicit α -adrenergic effects. Thus propranolol might actually assist α -adrenergic inhibition of the release of insulin from the β -cell. Propranolol would not alter the effects of parasympathetic stimulation in the pancreas. Therefore, an increased release of glucagon would still occur in the HEOD and propranolol-treated rat.

Experimentally, propranolol was found to have no significant effect on the HEOD-induced hyperglycemic response in the rat. As described above, this result would be expected on the basis of the model.

Atropine blocks muscarinic receptors (Innes & Nickerson, 1978). If as the model proposes, cholinergic stimulation, induced by HEOD, acts to elevate glucagon release, then atropine would prevent the HEOD-induced hyperglycemic response in the rat. Experimentally, atropine prevented the onset of hyperglycemia for a period of four hours. After this time, and despite additional atropine doses, HEOD-induced hyperglycemia was not inhibited. The antihyperglycemic effect of atropine was lost simultaneously with the onset of convulsive activity in our rats.

Stressful stimuli enhance sympathetic activity, which in turn stimulates the secretion of pancreatic glucagon while simultaneously inhibiting the release of insulin (Unger et al., 1978). Thus, as explained in the model, the loss of the antihyperglycemic effect of atropine is a simple conse-

quence of increased sympathetic activity.

β -adrenergic stimulation probably plays no role in the etiology of the normal HEOD-induced hyperglycemic response (see above discussion of propranolol experiment). However, such stimulation may be important in the HEOD-induced hyperglycemic response of the atropine-treated rat.

In summary, all our experimental results are consistent with the proposed model.

Ev. Alternative explanations.

The model postulates that the HEOD-induced hyperglycemic response results as a consequence of neurally-mediated alterations in normal pancreatic endocrine function. Kacew & Singhal (1974a, 1974b) believe the response results from a direct effect evoked in liver (i.e. pesticide-enhanced adenyl cyclase activity). Our model in contrast, assumes that liver biochemical function is largely unchanged by HEOD directly.

However, it is also possible that HEOD may act to alter normal neurological control of liver metabolism. A recent study by Seydoux et al. (1979) has shown that electrical stimulation of perivascular nerves of mouse liver will increase glucose production and release by the organ. This effect is inhibited by both α and β blocking agents (phentolamine and propranolol). However, the greatest inhibition was found with the α -blocker. These authors have suggested that rather than being controlled by circulating catecholamines and other hormones, carbohydrate metabolism at the level of the liver itself, may actually be subject to direct neural control.

Therefore, it is possible that even in the absence of elevated plasma glucagon, HEOD by increasing sympathetic stimulation of the liver may still be capable of inducing the hyperglycemic response.

Niijima (1981) has also recently reported the existence of glucose-sensitive afferents leading from the liver to the vagus in the mouse. This author states that elevated blood glucose levels in the portal vein inhibit neuronal activity in these afferents. Visceral afferent fibers also exit from the pancreas and follow the splanchnic nerves to the spinal cord (Woods & Porte, 1974). Niijima (1980) believes that these afferents along with other adrenal afferents are both reflexly affected by high blood glucose levels in the portal vein. Thus, high blood glucose concentration in portal vein blood appears to be correlated with decreased activity in these afferents. However, Woods & Porte (1974) believe the pancreatic afferents to be primarily concerned with mediating pain due to distension of the organ to the CNS.

If Niijima's view is correct, then hepatic, pancreatic and adrenal afferents might all play some role in the regulation of plasma glucose concentration. For example, do these afferents transmit information regarding blood glucose concentration to the hypothalamus? Does the hypothalamus utilize this information to modify neural activity in efferents leading back to the pancreas and liver? Finally, what, if any, is the effect of HEOD on the activity in these afferents and does HEOD alter integrative function in the hypothalamus?

Pancreatic secretory function is also influenced by many hormones. For example, somatostatin, somatotrophin, cortisol, adrenal catecholamines, gastrin and secretin all modify glucagon and insulin release (Unger et al., 1978). Nutrients such as glucose, amino-acids and free fatty acids also influence pancreatic function (Porte & Halter, 1982). In fact, plasma glucose concentration is generally held to be the primary regulator of pancreatic secretory function (Unger et al., 1978).

The proposed model assumes that in the HEOD-exposed animal, neurological as opposed to nutritional or humoral regulation of pancreatic function predominates. This would explain why hyperglycemia, for example, is unable to restore plasma glucose homeostasis in these animals. However, it is also possible that dieldrin may have some totally unexpected effect which could produce the same end result.

F. Conclusions.

Fi. Immature animals.

1. Dieldrin is more toxic to the immature than the mature rat.
2. Dieldrin induces only a transitory low-level hyperglycemia in the five day old rat. The pesticide does not alter plasma glucose concentration in the ten day old rat. Therefore, dieldrin is incapable of inducing an "adult-type" hyperglycemic response in the immature rat.
3. Glucose-induced hyperglycemia decreases the mortality rate in the dieldrin exposed immature rat. Thus the inability of the young rat to develop hyperglycemia in response to dieldrin-treatment could explain the enhanced toxicity of the compound in the young rat.

Fii. Mature animals.

1. Dieldrin induces a hyperglycemic response characterized by hyperglycemia and increased hepatic PEPCK activity in the mature rat.
2. Hepatic, fructose-1,6-diphosphatase activity, hepatic glycogen concentration and plasma free fatty acid levels are unaltered by dieldrin.
3. Insulin prevents dieldrin-induced hyperglycemia, causes hypoglycemia and increases the acute toxicity of dieldrin.
4. 2-Deoxyglucose increases dieldrin-induced hyperglycemia and by inference therefore, causes intracellular glucose deficiency in the CNS. It does not alter either the CNS hyperstimulation or the mortality caused by dieldrin.
5. Increasing dieldrin-induced hyperglycemia with exogenous d-glucose does not alter the acute toxicity of the insecticide. Phenobarbital attenuates the dieldrin-induced hyperglycemia and virtually abolishes the toxicity of dieldrin. Restoration of hyperglycemia with exogenous d-glucose in the phenobarbital and dieldrin-treated rat to a level equivalent to that seen for dieldrin treatment alone, restores the acute toxicity of the insecticide.

The above results indicate that the dieldrin-induced hyperglycemic response has no protective value in the mature rat.

6. Phenobarbital, atropine and L- α -methyldopa impair the ability of dieldrin to induce hyperglycemia. Propranolol is without effect.
Therefore, the dieldrin-induced hyperglycemic response is elicited by the central nervous system and is mediated via cholinergic and α -adrenergic pathways. However, the β -adrenergic system is not involved.

Fiii. Animals of all ages.

1. Dieldrin does not alter hepatic glucose-6-phosphatase activity.
2. Hepatic phosphoenolpyruvate carboxykinase activity is increased, three hours after exposure to dieldrin.

LITERATURE CITED

- Acosta, D., D.C. Anuforo, R. McMillin, W.H. Soine and R.V. Smith. (1979). "Comparision of Cytochrome P450 levels in Adult Rat Liver, Post-natal Rat Liver, and Primary Cultures of Post-natal Rat Hepatocytes." Life Sciences, 25: 1413-1418.
- Agarwal, N., S. Sanyal, G.K. Khuller, R.N. Chakravarti and D. Subrahmanyam. (1978). "Effect of acute administration of Dichlorodiphenyl Trichloroethane on certain enyzmes of Rhesus Monkey." Ind. J. Medical Res., 68: 1001-1006.
- Agarwal, N., S. Sanyal, G.K. Khuller, R.N. Chakravarti and D. Subramanyam. (1981). "Acute Exposure of Rhesus Monkeys to Dieldrin: Effect on Lipid Metabolism." Toxicol. & Appl. Pharmacol., 58: 100-104.
- Akkermans, L.M.A., J. Van den Bercken, and J.M. Van der Zalm. (1975a). "Effects of Aldrin-transdiol on Neuromuscular Facilitation and Depression." Eur. J. Parmacol., 31: 166-175.
- Akkermans, L.M.A., J. Van den Bercken, and M. Versluijs-Helder. (1975b). "Excitatory and Depressant Effects of Dieldrin and Aldrin-transdiol in the Spinal Cord of the Toad (Xenopus laevis)." Eur. J. Pharmacol., 34: 133-142.
- Akkermans, L.M.A., J.M. Van der Zalm and J. Van den Bercken. (1973). "Is Aldrin-transdiol the Active Form of the Insecticide Dieldrin?" Arch. Internationales de Pharmacodynamie et Therapie, 206: 363-364.
- Akkermans, L.M.A., J. Van den Bercken, J.M. Van der Zalm and H.W.M. Van Straaten. (1974). "Effects of Dieldrin (HEOD) and Some of Its Metabolites on Synaptic Transmission in the Frog Motor End-plate." Pest. Biochem., 4: 313-324.
- Aravindakshan, A., Bikram Chand, M. Ramachandran and Q.Z. Hussain. (1977). "Effect of Dietary DDT on Certain Biochemical Changes in Albino Rat." Ind. J. Experimen. Biol., 15: 578-579.
- Baginski, E.S., P.P. Foa and B. Zak. (1967). "Determination of Rat Liver Microsomal Glucose-6-Phosphatase Activity: Study of Citrate and Glucose-6-Phosphate Inhibition." Anal. Biochem., 21: 201-207.

- Baginski, E.S., P.P. Foa and B. Zak. (1974). "Glucose-6-Phosphatase." In H.U. Bergmeyer, ed. Methods of Enzymatic Analysis. 2nd edition. New York: Academic Press Inc. page: 876-880.
- Bailey, C.J. and P.R. Flatt. (1980). "Insulin and Glucagon during Pentobarbitone Anaesthesia." Diabete et Metabolisme (Paris), 6: 91-95.
- Bailey, C.J., C. Day, P.R. Flatt and T.W. Atkins. (1979). "Effects of Repeated Sodium Pentobarbitone Administration on Glucose Homeostasis in Mice." General Pharmacol., 11: 303-305.
- Ballard, F.J. (1970). "Carbohydrates." In U. Stave, ed. Physiology of the Perinatal Period: Vol. 1. Function and Biochemical Development in Mammals. New York: Appleton Century Crofts.
- Ballard, F.J. and R.W. Hanson. (1967). "Changes in Lipid Synthesis in Rat Liver during Development." Biochem. J., 102: 952-958.
- Ballard, F.J. and I.T. Oliver. (1962). "Appearance of Fructose-1,6-diphosphatase in Post-natal Rat Liver." Nature (Lond), 195: 498-499.
- Bandyopadhyay, S.K., R.K. Tiwari, A. Mitra, B. Mukherjee, A. Banerjee and G.C. Chatterjee. (1982a). "Effects of l-Ascorbic Acid Supplementation of Dieldrin Toxicity in Rats." Arch. Toxicol., 58: 227-232.
- Bandyopadhyay, S.K., R.K. Tiwari, A. Bhattacharyya and G.C. Chatterjee. (1982b). "Effect of Dieldrin on Rat Liver Plasma Membrane Enzymes." Toxicol. Letters, 11: 131-134.
- Bařon, R.L. and M.S. Walton. (1971). "Dynamics of HEOD (Dieldrin) in Adipose Tissue of the Rat." Toxicol. & Appl. Pharmacol., 18: 958-963.
- Basu, T.K., J.W. Dickerson and D.V.W. Parke. (1971). "Effect of Development on the Activity of Microsomal Drug-Metabolizing Enzymes in Rat Liver." Biochem. J., 124: 19-24.
- Beaudry, M.A., J.L. Chiasson and J.H. Exton. (1977). "Gluconeogenesis in the Suckling Rat." Amer. J. Physiol., 233: E175-E180.
- Bergman, R.N. and R.E. Miller. (1973). "Direct Enhancement of Insulin Secretion by Vagal Stimulation of the Isolated Pancreas." Amer. J. Physiol., 225: 481-486.

- Bergmeyer, H.U., E. Bernt, M. Grassl and G. Michal. (1974). "Evaluation of experimental results." In H.U. Bergmeyer, ed. Methods of Enzymatic Analysis. New York and London: Academic Press Inc. pages: 388-317.
- Bhatia, S.C. and T.A. Venkitasubramanian. (1972). "Mechanism of Dieldrin-Induced Fat Accumulation in Rat Liver." J. Agricultural Food Chem., 20: 993-996.
- Bhatia, S.C., S.C. Sharma, V.N. Damodaran and T.A. Venkitasubramanian. (1971). "Changes in Rat Ascorbic Acid Status and Adrenocortical Activity during Acute Dieldrin Toxicity." Ind. J. Biochem. & Biophysics, 8: 57-58.
- Bhatia, S.C., S.C. Sharma and T.A. Venkitasubramanian. (1972). "Acute Dieldrin Toxicity: Biochemical Changes in the Blood." Arch. Env. Health, 24: 369-372.
- Bhatia, S.C., S.C. Sharma and T.A. Venkitasubramanian. (1973). "Effect of Dieldrin on Hepatic Carbohydrate Metabolism and Protein Biosynthesis in vivo." Toxicol. & Appl. Pharmacol., 24: 216-229.
- Bloom, S.R., A.V. Edwards and J.A. Vaughan. (1973). "The role of the Sympathetic Innervation in the Control of Plasma Glucagon Concentration in the Calf." J. Physiol. (Lond.), 233: 457-466.
- Bloom, S.R., A.V. Edwards and N.J.A. Vaughan. (1974). "The role of the Autonomic Innervation in the Control of Glucagon Release during Hypoglycemia in the Calf." J. Physiol. (Lond.), 236: 611-623.
- Boyd, E.M. (1973). "Glucose." - The Toxicity of Pure Foods. Cleveland, Ohio: C.R.C. Press.
- Butler, T.C., D. Mahaffee and C. Mahaffee. (1952). "The Role of the Liver in the Metabolic Disposition of Mephobarbital." J. Pharmacol. Exp. Therapeutics, 186: 364-369.
- Cegrell, L. (1968). "Adrenergic Nerves and Monoamine-containing Cells in the Mammalian Endocrine Pancreas." Acta Physiol. Scand. 73, Suppl., 314: 5-60.
- Chipman, J.K. and C.H. Walker. (1979). "The Metabolism of Dieldrin and Two of Its Analogues: The Relationship between Rates of Microsomal Metabolism and Rates of Excretion of Metabolites in the Male Rat." Biochem. Pharmacol., 28: 1337-1345.

- Costella, J.C. and B.B. Virgo. (1980). "Is Dieldrin-Induced Congenital Inviability Mediated by CNS Hyperstimulation or Altered Carbohydrate Metabolism?" Can. J. Physiol. & Pharmacol., 58: 633-637.
- Crammer, F.B. and G.A. Neville. (1953). "A colorimetric Method for Estimating 2-Deoxyglucose." J. Franklin Inst., 254: 379-381.
- Daniel, P.M. and J.R. Henderson. (1967). "The Effect of Vagal Stimulation on Plasma Insulin and Glucose Levels in the Baboon." J. Physiol. (Lond.), 192: 317-327.
- Domino, E.F. (1962). "Sites of Action of Some Central Nervous System Depressants." Ann. Reviews Pharmacol., 2: 215-258.
- Doull, J. (1976). "The treatment of Insecticide Poisoning" In C.F. Wilkinson, ed. Insecticide Biochemistry and Physiology. London, New York: Plenum Press.
- Dudeja, P.K., S.N. Sanyal, N. Agarwal, T.N. Roa, D. Subrahmanyam and G.K. Khuller. (1980). "Acute Exposure of Rhesus Monkeys to DDT: Effect on Carbohydrate Metabolism." Chem-Biol. Interact., 31: 203-208.
- Ensinck, J.W. and R.H. Williams. (1981). "Disorders causing Hypoglycemia." In R.H. Williams, ed. Textbook of Endocrinology. 6th edition. London, Toronto: W.B. Saunders Company.
- Esterhuizen, A.C. and S.L. Howell. (1970). "Ultrastructure of the A-cells of Cat Islets of Langerhans following Sympathetic Stimulation of Glucagon Secretion." J. Cell Biol., 46: 593-631.
- Fallon, H.J. and E.L. Kemp. (1968). "Effect of Diet on Hepatic Triglyceride Synthesis." J. Clin. Invest., 47: 712-719.
- Feldman, S., C.S. Van der Heide and R.W. Porter. (1959). "Evoked Potentials in the Hypothalamus." Amer. J. Physiol., 196: 1163-1167.
- Fitzhugh, O.G. and A.A. Nelson. (1947). "The chronic Oral Toxicity of DDT (2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane)." J. Pharmacol. & Exp. Therapeutics, 89: 18-30.
- Frohman, L.A. and L.L. Bernardis. (1971). "Effect of Hypothalamic Stimulation on Plasma Glucose, Insulin and Glucagon Levels." Amer. J. Physiol., 221: 1596-1603.

- Frohman, L.A., L.L. Bernardis and M.E. Stachura. (1974). "Factors Modifying Plasma Insulin and Glucose Responses to Ventromedial Hypothalamic Stimulation." Metabol. 23: 1847-1857.
- Frohman, L.A., E.Z. Ezdinli and R. Javid. (1967). "Effect of Vagotomy and Vagal Stimulation on Insulin Secretion." Diabetes, 16: 443-448.
- Gil-Ad, I., G. Udeschini, D. Cocchi and E.E. Muller. (1975). "Hyporesponsiveness to Glucoprivation during Postnatal Period in the Rat." Amer. J. Physiol. 229: 512-517.
- Gerich, J.E., M.A. Charles and G.M. Grodsky. (1976). "Regulation of Pancreatic Insulin and Glucagon Secretion." Ann. Reviews Physiol. 38: 353-388.
- Goldenthal, E.I. (1971). "A Compilation of LD50 Values in Newborn and Adult Animals." Toxicol. & Appl. Pharmacol. 18: 185-207.
- Gowdy, C.W. and G.W. Stavsky. (1955). "A Study of the Autonomic Manifestations seen in Acute Aldrin and Dieldrin Poisoning." Can. J. Biochem. Physiol. 33: 272-282.
- Gowdy, C.W., A.R. Graham, J.J. Sequin and G.W. Stavsky. (1954). "The Pharmacological Properties of the Insecticide Dieldrin." Can. J. Biochem. Physiol. 32: 498-504.
- Greene, E.C. (1955). "Anatomy of the Rat. Digestive Tract and Associated Glands." Transact. Amer. Philosophical Soc. 27: 90-107.
- Greengard, O. (1969). "The Hormonal Regulation of Enzymes in Prenatal and Postnatal Rat Liver." Biochem. J. 115: 19-23.
- Grodsky, G.M. (1977). "The Chemistry and Function of the Hormones." In H.A. Harper, U.W. Rodwell and P.A. Mayes editors. Review of Physiological Chemistry, 16th edition. Los Altos, Ca.: Lange Medical Publications.
- Hahn, P. (1970). "Lipids." In U. Stave ed. Physiology of the Perinatal Period. Vol. 1. Function and Biochemical Development in Mammals. New York: Appleton, Century, Crofts.
- Hahn, P., R. Greenberg, M. Dobiasova and Z. Drahota. (1968). "Triglyceride Synthesis from Various Precursors in Adipose Tissue of the Rat during Development." Can. J. Biochem. 46: 735-741.

- Hall, S.E.H., A.J. Hall, R.A. Layberry, M. Berman and G. Hetenyi jr. (1976). "Effects of Age and Fasting on Gluconeogenesis from Glycerol in Dogs." Amer. J. Physiol., 230: 362-367.
- Halmagyi, D.R.J., I.R. Neering, L. Lazarus, J.D. Young and J. Pullin. (1969). "Plasma Glucose in Experimental Post-hemorrhagic Shock." J. Trauma, 9: 328-326.
- Hartshorn, R.D., L.M. Demers, L.G. Sultatos, E.S. Vesell, C.M. Lang and H.C. Hughes Jr. (1979). "Effects of Chronic Parenteral Carbohydrate Administration on Hepatic Drug Metabolism in the Rat." Pharmacol., 18: 103-111.
- Hayes jr., W.J. (1974). "Distribution of Dieldrin Following a Single Oral Dose." Toxicol. & Appl. Pharmacol., 28: 485-492.
- Heath, D.F. and M. Vandekar. (1964). "Toxicity and Metabolism of Dieldrin in Rats." Brit. J. Industr. Med., 21: 269-279.
- Henderson, G.L. and D.E. Woolley. (1969). "Studies on the Relative Insensitivity of the Immature Rat to the Neurotoxic Effects of 1,1,1-Trichloro 2,2-bis(p-chlorophenyl) ethane (DDT)." J. Pharmacol. & Exp. Therapeutics, 170: 173-188.
- Henning, M. (1969). "Studies on the Mode of Action of α -Methyldopa." Acta Physiol. Scand. Suppl., 322: 1-37.
- Herrman, J.L. and R.C. Nordlie. (1972). "Hepatic Carbamyl Phosphate: Glucose Phosphotransferase Distribution, Comparative Kinetics, and Responses to Alloxan- or Mannoheptulose-Induced Diabetes." Arch. Biochem. Biophysics, 152: 180-186.
- Hetenyi, G. and J.S. Cowan. (1988). "Glucoregulation in the Newborn." Can. J. Physiol. & Pharmacol., 58: 879-888.
- Hickenbottom, J.P. and E.T. Yau. (1974). "Effects of DDT on Liver Glycogen Metabolism in Rats." Pharmacologist, 16: 35.
- Hiddemen, J.W. and H.H. Cornish. (1978). "Dieldrin Induced Hyperglycemia in Rats." Toxicol. & Appl. Pharmacol., 17: 277-278.
- Hodge, H.C., A.M. Boyce, W.B. Deichmann and H.E. Kraybill. (1967). "Toxicology and No-effect Levels of Aldrin and Dieldrin." Toxicol. & Appl. Pharmacol., 10: 613-675.

- Houpt, T.R. and H.E. Hance. (1977). "Threshold Levels of 2-deoxy-d-glucose for the Hyperglycemic Response: Dog and Goat Compared." Life Sciences, 21: 513-518.
- Hutson, D.H. (1976). "Comparative Metabolism of Dieldrin in the Rat (CFE) and in Two Strains of Mouse (CF1 and LAC6)." Food & Cosmetic Toxicol., 14: 577-591.
- Innes, I.R. and M. Nickerson. (1978). "Drugs Inhibiting the Action of Acetylcholine on Structures Innervated by Postganglionic Parasympathetic Nerves (antimuscarinic drugs)." In L.S. Goodman and A. Gilman, editors. The Pharmacological Basis of Therapeutics, 4th edition. London, Toronto: Collier-Macmillan Ltd.
- Iturri, S.J. and D. Wolff. (1981). "Inhibition of the Active Transport of d-glucose and l-tyrosine by DDT and DDE in the Rat Small Intestine." Comp. Biochem. Physiol., 71C: 131-134.
- Iversen, J. and M.D. Arhus. (1973). "Effect of Acetylcholine on the Secretion of Glucagon and Insulin from the Isolated, Perfused Canine Pancreas." Diabetes, 22: 381-387.
- Joy, R.M. (1977). "Contrasting Actions of Dieldrin and Aldrin-transdiol, Its Metabolite, on Cat CNS Function." Toxicol. & Appl. Pharmacol., 42: 137-148.
- Kacew, S. and R.L. Singhal. (1973a). "Adaptive Response of Hepatic Carbohydrate Metabolism to Oral Administration of p,p'-1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane in Rats." Biochemical Pharmacol., 22: 47-57.
- Kacew, S. and R.L. Singhal. (1973b). "The Influence of pp'-DDT, Chlordane, Heptachlor and Endrin on Hepatic and Renal Carbohydrate Metabolism and cyclic AMP - adenylyl cyclase system." Life Science, 13: 1363-1371.
- Kacew, S. and R.L. Singhal. (1973c). "Stimulation of Kidney Cortex cyclic 3'5'Adenosine monophosphate-H³ Formation by DDT and Related Insecticides." Fed. Proc. Fed. Amer. Soc. Exp. Biol., 32: 802.
- Kacew, S. and R.L. Singhal. (1973d). "Metabolic Alterations after Chronic Exposure to α -Chlordane." Toxicol. & Appl. Pharmacol., 24: 539-544.
- Kacew, S. and R. Singhal. (1974a). "Role of cyclic Adenosine 3':5' Monophosphate in the action of 1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane (DDT) on Hepatic and Renal Metabolism." Biochem. J., 142: 145-152.

- Kacew, S. and R.L. Singhal. (1974b). "Effect of Certain Halogenated Hydrocarbon Insecticides on cyclic Adenosine 3'5'Monophosphate- H^3 Formation by Rat Kidney Cortex." J. Pharmacol. & Exp. Therapeutics, 188: 265-276.
- Kacew, S., R.L. Singhal, P.D. Hrdina and G.M. Ling. (1972a). "Changes in kidney Cortex Gluconeogenic Enzymes Induced by 1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane (DDT)." J. Pharmacol. & Exp. Therapeutics, 181: 234-243.
- Kacew, S., R.L. Singhal and G.M. Ling. (1972b). "DDT-Induced Stimulation of Key Gluconeogenic Enzymes in Rat Kidney Cortex." Can. J. Biochem., 50: 225-229.
- Kacew, S., D.J.B. Sutherland and R. Singhal. (1973). "Biochemical changes following Chronic Administration of Heptachlor, Heptachlor epoxide and Endrin to male rats." Environ. Physiol. Biochem., 3: 221-229.
- Kaneto, A., K. Kosaka and K. Nakao. (1967). "Effects of Stimulation of the Vagus Nerve on Insulin Secretion." Endocrinol., 80: 530-536.
- Kaneto, A., E. Miki and K. Kosaka. (1974). "Effects of Vagal Stimulation on Glucagon and Insulin Secretion." Endocrinol., 95: 1005-1009.
- Kingsley, G.R. and G. Getchell. (1960). "Direct Ultramicro Glucose Oxidase Method for Determination of Glucose in Biological Fluids." Clin. Chem., 5: 466-475.
- Kohli, K.K., S.C. Bhatia and T.A. Venkitasubramanian. (1975a). "Effect of Dieldrin Toxicity on Acetate and Palmitate Metabolism in Rat Liver." Environ. Physiol. Biochem., 5: 119-125.
- Kohli, K.K., S.C. Sharma, S.C. Bhatia and T.A. Venkitasubramanian. (1975b). "Biochemical Effects of Chlorinated Insecticides: DDT and Dieldrin." J. Scientific Indust. Res., 34: 462-470.
- Korte, F. and H. Arent. (1965). "Metabolism of Insecticides, IX (1) Isolation and Identification of Dieldrin Metabolites from Urine of Rabbits after Oral Administration of Dieldrin- ^{14}C ." Life Sciences, 4: 2017-2026.
- Krough, M.E. (ed.). (1981). Compendium of Pharmaceuticals and Specialties, 16th edition. Canadian Pharmaceutical Association. Toronto: Southam Murry.

- Kwan, K.C., E.L. Foltz, G.O. Breault, J.E. Baer and J.A. Totaro. (1976). "Pharmacokinetics of Methlydopa in Man." J. Pharmacol. & Exp. Therapeutics, 198: 264-277.
- Latzo, E. and M. Gibbs. (1974). "Alkaline C1-fructose-1,6-diphosphatase." In W.V. Bergmeyer ed. Methods of Enzymatic Analysis. 2nd edition. New York: Academic Press Inc. pages: 881-884.
- Lindsey, C.A., G.R. Faloon and R.H. Dobbs. (1975). "Plasma Glucagon levels during rapid Exsanguination with and without Adrenergic Blockade." Diabetes, 24: 313-316.
- Lindstrom, F.T., J.W. Gillett and S.E. Rodecap. (1974). "Distribution of HEOD (Dieldrin) in Mammals: I. Preliminary Model." Arch. Environm. Contamin. & Toxicol., 2: 9-42.
- Litchfield, J.T. and F.J. Wilcoxon. (1949). "A Simplified Method for Evaluating Dose-effect Experiments." J. Pharmacol. & Exp. Therapeutics, 96: 99-113.
- Loomis, T.A. (1978). "The Basis of Antidotal Therapy." - Essentials of Toxicology. Philadelphia: Lea & Febiger.
- Loubatieres, A., M.M. Mariani and J. Chapel. (1970). "Etude sur le pancreas isole et perfuse du rat, de l'action des recepteurs cholinergiques sur l'insulino-secretion." Compt. Rend. Soc. Biol., 164: 2345-2352.
- Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall. (1951). "Protein Measurement with Folin Phenol Reagent." J. Biol. Chem., 193: 265-275.
- Lu, F.C., D.C. Jessup and A. Lavallee. (1965). "Toxicity of Pesticides in Young Rats versus Old Rats." Food and Cosmet. Toxicol., 3: 591-596.
- Madvig, P. and S. Abraham. (1980). "Relationship of Malic Enzyme Activity to Fatty Acid Synthesis and the Pathways of Glucose Catabolism in Developing Rat Liver." J. Nutrit., 110: 98-99.
- Mahmood, A., N. Agarwal, S. Sanyal, P.K. Dudeja and D. Subrahmanyam. (1981). "Acute Dieldrin Toxicity: Effect on the Uptake of Glucose and Leucine and on Brush Border Enzymes in Monkey Intestine." Chem-Biol. Interact., 37: 165-170.
- Malaisse, W., F. Malaisse-Lagae, P.H. Wright and J. Ashmore. (1967). "Effects of Adrenergic and Cholinergic Agents Upon Insulin Secretion." Endocrinol., 80: 975-978.

- Marliss, E.B., L. Girardier, J. Seydoux, Y. Kanazawa, C. Wollheim, L. Orci and D. Porte Jr. (1972). "Glucagon Release by Pancreatic nerve stimulation: Further evidence for direct neural control of endocrine pancreatic secretion." Europ. J. Clin. Invest., 2: 295-296.
- Marliss, E.B., L. Girardier, J. Seydoux, C.B. Wollheim, Y. Kanazawa, L. Orci, A.E. Renold and D. Porte Jr. (1973). "Glucagon Release Induced by Pancreatic Nerve Stimulation in the Dog". J. Clin. Invest., 52: 1246-1259.
- Matthews, H.B. and J.D. McKinney. (1974). "Dieldrin Metabolism to Cis-dihydroaldrindiol and Epimerization of Cis to Trans-dihydroaldrindiol by Rat Liver Microsomes." Drug Metabol. & Disposition, 2: 333-340.
- Matthews, H.B., J.D. McKinney and G.W. Lucier. (1971). "Dieldrin Metabolism, Excretion and Storage in Male and Female Rats." Agricult. & Food Chem., 19: 1244-1248.
- Mayes, P.A. (1977a). "The Citric Acid Cycle." In H.A. Harper, U.W. Rodwell and P.A. Mayes, editors. Review of Physiological Chemistry, 16th edition. Los Altos, Ca.: Lange Medical Publications.
- Mayes, P.P. (1977b). "Metabolism of Carbohydrate." In H.A. Harper, U.W. Rodwell and P.A. Mayes, editors. Review of Physiological Chemistry, 16th edition. Los Altos, Ca.: Lange Medical Publications.
- McKinney, J.D., H.B. Matthews and L. Fishbein. (1972). "Major Fecal Metabolite of Dieldrin in Rat. Structure and Chemistry." Agricult. & Food Chem., 20: 597-602.
- Merkens, L.S., H.M. Tepperman and J. Tepperman. (1980.) "Effects of Short-term Dietary Glucose and Fructose on Rat Serum Triglyceride Concentration." J. Nutrit., 110: 982-988.
- Moore, T.J., A.P. Lione, D.M. Regen, H.L. Tarpley and P.L. Raines. (1971). "Brain Glucose Metabolism in the Newborn Rat." Amer. J. Physiol., 221: 1746-1753.
- Morgan, C.R. and R.T. Lobl. (1966). "A Histochemical Study of Neuroinsular Complexes in the Pancreas of the Rat." Anat. Record, 160: 231-238.
- Murphy, S.D. (1980). "Pesticides." In J. Doull, C.D. Klaassen, and M.O. Amdur, editors. Casarett and Doull's Toxicology: The Basic Science of Poisons, 2nd edition. New York, Toronto: Macmillan Publishing Co., Inc.

- Nickerson, M. (1970). "Drugs Inhibiting Adrenergic Nerves and Structures Innervated by them. II.: β -Adrenergic Blocking Agents." In L.S. Goodman and A. Gilman, editors. The Pharmacological Basis of Therapeutics, 4th edition. London, Toronto: Collier-Macmillan Ltd.
- Niiijima, A. (1980). "Glucose-sensitive Afferent Nerve Fibers in the Liver and Regulation of Blood Glucose." Brain Res. Bull., 5 (Suppl. 4): 175-179.
- Niiijima, A. (1981). "Neurophysiological Evidence for Hepatic Glucose-sensitive Afferents: Commentary on "The Current Status of Hepatic Theory of Food Intake Control".
Appetite, 2: 151-152.
- O'Brien, R.D. (1967). "Cyclodienes." - Insecticides: Action and Metabolism. New York, London: Academic Press.
- Pasha, S.T. (1981). "Changes in Hepatic Microsomal Enzymes due to DDT, DDE and DDD Feeding in CF-1 mice." Ind. J. Med. Res., 17: 926-931.
- Pegorier, J.P., A. Leturque, P. Ferre, P. Turlan and J. Girard. (1983). "Effects of medium-chain Triglyceride feeding on Glucose Homeostasis in the Newborn Rat." Amer. J. Physiol., 244: E329-E334.
- Petrescu, I., O. Bojan, M. Saied, O. Barzu, F. Schmidt and H.F. Kuhnle. (1979). "Determination of Phosphoenolpyruvate Carboxykinase Activity with Deoxyguanosine-5'-diphosphate as Nucleotide Substrate." Anal. Biochem., 26: 279-281.
- Platt, D.S. and B.L. Cöckerill. (1969). "Biochemical Changes in Rat Liver in response to Treatment with Drugs and other Agents. II. Effects of halothane, DDT, other chlorinated hydrocarbons, thioacetamide, dimethylnitrosoamine and ethionine." Biochem. Pharmacol., 18: 445-457.
- Porte Jr., D. and J.B. Halter. (1982). "The Endocrine Pancreas and Diabetes Mellitus." In R.H. Williams, ed. Textbook of Endocrinology, 6th edition. London, Toronto: W.B. Saunders Company.
- Porte Jr., D., P.H. Smith and J.W. Ensink. (1976). "Neurohumoral Regulation of the Pancreatic α and β cells." Metabol., 25: 1435-1456.
- Porte Jr., D., L. Girardier, J. Seydoux, Y. Kanazawa and J. Posternak. (1973). "Neural Regulation of Insulin Secretion in the Dog." J. Clin. Invest., 52: 210-214.

- Poschl, E., K. Buff and J. Berndt. (1982). "Effect of DDT on Adenyl Cyclase Activity in Rat Liver Cell Membranes." Chem-Biol. Interact., 39: 369-374.
- Pylkko, O.O. and D.M. Woodbury. (1961). "The Effect of Maturation on Chemically Induced Seizures in Rats." J. Pharmacol. & Exp. Therapeutics, 131: 185-190.
- Rao, N.T., S. Sanyal, N. Agarwal and D. Subrahmanyam. (1981). "Effect of a Single Oral Dose of DDT on Lipid Metabolism in Rat." Toxicol. Letters, 7: 263-266.
- Robertson, R.P. and D. Porte jr. (1973a). "Adrenergic Modulation of Basal Insulin Secretion in Man." Diabetes, 22: 1-8.
- Robertson, R.P. and D. Porte jr. (1973b). "The Glucose Receptor: A defective mechanism in Diabetes Mellitus distinct from the beta-adrenergic receptor." J. Clin. Invest., 52: 870-876.
- Robinson, J., M. Roberts, M. Baldwin and A.I.T. Walker. (1969). "The Pharmacokinetics of HEOD (Dieldrin) in the Rat." Food and Cosmet. Toxicol., 7: 317-332.
- Rohlf, F.J. and R.R. Sokal. (1969). "Statistical Tables." San Francisco: W.H. Freedman and Company.
- Samols, E. and G.C. Weir. (1979). "Adrenergic Modulation of Pancreatic α , β , and D cells: α -Adrenergic Suppression and β -Adrenergic Stimulation of Somatostatin Secretion, α -Adrenergic Stimulation of Glucagon Secretion in the Perfused Dog Pancreas." J. Clin. Invest., 63: 230-238.
- Schneck, D.W., J.F. Pritchard and A.H. Hayes jr. (1977). "Studies on the Uptake and Binding of Propranolol by Rat Tissues." J. Pharmacol. & Exp. Therapeutics, 203: 621-629.
- Schroeder, M.E., D.L. Shankland and R.M. Hollingworth. (1977). "The Effects of Dieldrin and Isomeric Aldrin Diols on Synaptic Transmission in the American Cockroach and Their Relevance to the Dieldrin Poisoning Syndrome." Pest. Biochem. & Physiol., 7: 403-415.
- Seifter, S., S. Dayton, B. Novic and E. Muntwyler. (1950). "Glycogen Determination by Use of the Anthrone Reagent." Arch. Biochem., 25: 191-200.
- Seydoux, J., M.J.A. Brunsmann, B. Jeanrenard and L. Girardier. (1979). " α -Sympathetic Control of Glucose Output of Mouse Liver Perfused in situ." Amer. J. Physiol., 236: E323-E327.

- Sharpless, S.K. (1970). "Hypnotics and Sedatives. I. The Barbiturates." In L.S. Goodman and A. Gilman, editors. The Pharmacological Basis of Therapeutics, 4th edition. London, Toronto: Collier-Macmillan Ltd.
- Singhal, R.L., J.R.E. Valadares and W.S. Schwark. (1970). "Metabolic Control Mechanisms in Mammalian Systems IX: Estrogen like Stimulation of Uterine Enzymes by α -1,1,1-Trichloro-2,2-bis(p-chlorophenyl) Ethane." Biochem. Pharmacol., 19: 2145-2155.
- Smith, P.H. and D. Porte Jr. (1976). "Neuropharmacology of the Pancreatic Islets." Ann. Review Pharmacol & Toxicol., 16: 269-285.
- Sokal, R.R. and J.J. Rohlf. (1969). "Biometry: The principles and practise of statistics in biological research." San Francisco: W.H. Freedman and Company.
- Soloni, F.G. and L.C. Sardina. (1973). "Colorimetric Micro-determination of Free Fatty Acids." Clin. Chem., 19: 419-424.
- Srivastava, A.K. and N.N. Singh. (1981). "Effect of Aldrin on Carbohydrate Metabolism in Indian Catfish." Acta Pharmacol. Toxicol., 49: 266-269.
- Steel, R.G.D. and J.H. Torrie. (1960). "Principles and Procedures of Statistics, with special reference to the biological sciences." New York, London: McGraw-Hill Book Company Inc.
- Steffens, A.B., G.J. Mogenson and J.A.F. Stevenson. (1972). "Blood Glucose, Insulin and Free Fatty Acids after Stimulation and Lesion of the Hypothalamus." Amer. J. Physiol., 222: 1446-1452.
- St. Omer, V.V. (1970). "Chronic and Acute Toxicity of the Chlorinated Hydrocarbon Insecticides in Mammals and Birds." Can. Vet. J., 11: 215-226.
- Stohlman, E.F. and R.D. Lillie. (1948). "The Effect of DDT on the Blood Sugar and of Glucose Administration on the Acute and Chronic Poisoning of DDT in rabbits." J. Pharmacol. & Exp. Therapeutics, 32: 351-361.
- Story, D.L. and R.A. Freedland. (1978a). "Decreased Glucagon and cAMP Stimulation of Gluconeogenesis from Lactate in Isolated Hepatocytes from Rats fed α -DDT." Fed. Proc. Amer. Fed. Soc. Exp. Biol. 37(3): 505.

- Story, D.L. and R.A. Freedland. (1978b). "The Effect of DDT feeding on Gluconeogenesis in Isolated Hepatocytes from starved Rats." Toxicol. & Appl. Pharmacol., 43: 547-557.
- Story, D.L. and R.A. Freedland. (1979). "The Effect of op-DDT Feeding on the Hormonal Stimulation of Gluconeogenesis from Lactate in Isolated Hepatocytes from 48hr Starved Rats." Toxicol. & Appl. Pharmacol., 48: 435-443.
- Story, D.L., P.E. Russell and R.A. Freedland. (1976). "Effects of feeding DDT on Gluconeogenesis in Rat Liver." Fed. Proc. Fed. Amer. Soc. Exp. Biol., 35: 504.
- Story, D.L., K.C. Triebwasser and R.A. Freedland. (1982). "Nitrogen metabolism in op-DDT-Fed Rats." Toxicol. & Appl. Pharmacol., 63: 275-280.
- Stumpf, B. and H. Kraus. (1979). "Regulatory Aspects of Glucose and Ketone Body Metabolism in Infant Rat Brain." Pediatric Res., 13: 585-590.
- Taylor, C.B., E. Bailey and W. Bartley. (1967). "Changes in Hepatic Lipogenesis during Development of the Rat." Biochem. J., 105: 717-722.
- Turlan, P., P. Ferre and J.R. Girard. (1983). "Evidence that Medium-chain Fatty Acid Oxidation Can Support an Active Gluconeogenesis in the Suckling Newborn Rat." Biol. Neonate, 43: 103-108.
- Underwood, A.H. and E.A. Newsholme. (1965). "Some Properties of Fructose-1,6-Diphosphatase of Rat Liver and their relation to the Control of Gluconeogenesis." Biochem. J., 95: 767-774.
- Unger, R.H., R.E. Dobbs and L. Orci. (1978). "Insulin, Glucagon and Somatostatin Secretion in the Regulation of Metabolism." Ann. Review Physiol., 40: 307-343.
- U.S. Pharmacopeial Convention, Inc. (1975). United States Pharmacopeia. 19th revision. Easton, Pa.: Mack Publishing Co.
- Uretsky, N.J. and L.S. Seiden. (1969). "Effect of α -Methyl dopa on the Reserpine-induced Suppression of Motor Activity and the Conditioned Avoidance Response." J. Pharmacol. & Exp. Therapeutics, 168: 153-162.
- Van den Berken, J. and T. Narahashi. (1974). "Effects of Aldrin-transdiol - a metabolite of the insecticide dieldrin on nerve membrane." Eur. J. Pharmacol., 27: 255-258.

- Vaughan, N.J.A., S.R. Bloom O. Ogarwa, P.M.M. Bircham and A.V. Edwards. (1973). "The role of the Autonomic Nervous system in the Control of Glucagon Release during Hypoglycemia in the Calf." Experientia, 29: 805-806.
- Vernon, R.G. and D.G. Walker. (1972). "Glucose Metabolism in the Developing Rat: Studies in vivo." Biochem. J. 127: 521-529.
- Wang, C.M., T. Narahashi and M. Yamada. (1971). "The Neurotoxic Action of Dieldrin and Its Derivatives in the Cockroach." Pest. Biochem. & Physiol. 1: 84-91.
- Weiner, N. (1980). "Drugs that Inhibit Adrenergic Nerves and Block Adrenergic Receptors." In A.G. Gilman and L.S. Goodman, editors. The Pharmacological Basis of Therapeutics, 6th. edition. New York, Toronto: Macmillan Publishing Co. Inc.
- Weiss, H.R., J.A. Lipp, J.A. Neubauer and R.S. Feldman. (1976). "Effect of Alpha and Beta Adrenergic Blockade on Oxygen Transport in Rat Skeletal Muscle and Brain." J. Pharmacol. & Exp. Therapeutics, 198: 403-411.
- White, A., P.H. Handler, E.L. Smith, R.L. Hill and I.R. Lehman. (1978). "Carbohydrate Metabolism I." - Principles of Biochemistry, 6th. edition. New York, Toronto: McGraw-Hill Book Company.
- Wilhjelm, B. and H. Langgard. (1968). "Effects of Phenobarbital, Phenytoin and Anoxia on Connective Tissue in Mice." Acta Neurol. Scand. 44: 643-648.
- Woods, S.C. and D. Porte Jr. (1974). "Neural Control of the Endocrine Pancreas." Physiol. Reviews, 54: 596-619.
- Yau, E.T. and J.H. Mennear. (1977). "The Inhibitory Effect of DDT on Insulin Secretion in Mice." Toxicol. & Appl. Pharmacol. 39: 81-88.
- Yeung, D. and I.T. Oliver. (1967). "Gluconeogenesis from Amino Acids in Neonatal Rat Liver." Biochem. J. 103: 744-748.

APPENDICES.

Appendix 1. List of chemicals and suppliers.

A. Chemicals (by assay or preparation)

Ai. 2-deoxyglucose (plasma) assay

2-deoxyglucose - Sigma
3,5-diamino-benzoic acid dihydrochloride - Aldrich
phosphoric acid - Fisher
sodium hydroxide - Fisher
zinc sulphate - Fisher

Aii. Free fatty acid (plasma) assay

acetone - Fisher
albumin (BSA) - Sigma
ammonium hydroxide - Fisher
chloroform - Canlab (Baker)
copper nitrate ($\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$) - Fisher
isopropanol (2-propanol) - Fisher
methanol - Fisher
nitrogen - Gas Dynamics
oxalic acid bis-(cyclohexylidenehydrazide) - Aldrich
palmitic acid (hexadecanoic acid) - Sigma
sodium azide - Fisher
sodium hydroxide - Fisher
triethanolamine - Aldrich

Aiii. Fructose-1,6-diphosphatase (hepatic) assay

fructose-1,6-diphosphate (trisodium salt) - Sigma
fructose-1,6-diphosphatase
(EC # 3.1.3.11) 5 U/mg - Sigma
glucose-6-phosphate dehydrogenase
(EC # 1.1.1.49) 245 U/mg - Sigma
hydrochloric acid - Fisher
magnesium chloride-6-hydrate - Canlab (Baker)
2-mercaptoethanol - Sigma
NADP (β -nicotinamide adenosine dinucleotide reduced)
disodium salt grade III - Sigma
phosphoglucose isomerase
(EC # 5.3.1.9) 750 U/mg - Sigma
sucrose (grade I) - Sigma
trizma base (tris-[hydroxymethyl]aminomethane) - Sigma

Aiv. Glucose (plasma) assay

benzoic acid - Fisher
dianisidine-HCl - Sigma

d-glucose - Fisher
 glucose oxidase (EC # 1.1.3.4) 15000 U/g - Sigma
 horseradish peroxidase
 (EC # 1.11.1.7) 120 U/mg - Sigma
 potassium phosphate (KH_2PO_4) - Fisher
 sodium hydroxide - Fisher
 sulfuric acid - Fisher

Av. Glucose-6-phosphatase (hepatic) assay

acetic acid - Eastman Kodak
 ammonium molybdate - Canlab (Baker)
 L-ascorbic acid - Fisher
 cacodylic acid (dimethyl-arsinic acid)
 free acid form - Sigma
 EDTA- Na_2
 (disodium ethylenediamine tetra-acetate) - Fisher
 glucose-6-phosphate monosodium salt - Sigma
 glucose-6-phosphatase
 (EC # 3.1.3.9) 0.025 U/mg - Sigma
 potassium phosphate (K_2HPO_4) - Fisher
 sodium arsenite - Fisher
 sodium citrate - Fisher
 trichloroacetic acid - Fisher

Avi. Glycogen (hepatic) assay

anthrone - Fisher
 ethanol - Commercial Alcohols
 glycogen (rabbit) - Sigma
 potassium hydroxide - Canlab (Baker)
 sulfuric acid - Fisher

Avii. Phosphoenolpyruvate carboxykinase (hepatic) assay

carbon dioxide (CO_2 : 4.9% O_2 : balance) - Gas Dynamics
 GDP (guanosine-5'-diphosphate) sodium salt type I -
 Sigma
 hydrochloric acid - Fisher
 malate dehydrogenase (EC #1.1.1.37) - Sigma
 manganese chloride - Sigma
 NADH (nicotinamide dinucleotide reduced) - Sigma
 phosphoenolpyruvic acid (tri-monocyclohexylammonium
 salt) - Sigma
 sodium bicarbonate - Fisher
 sucrose (grade I) - Sigma
 trizma base (tris-[hydroxymethylaminomethane]) - Sigma

Aviii. Protein = Lowry (hepatic) assay

albumin (BSA) - Sigma.
copper sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) - Fisher
phenol reagent 2N (Folin-Ciocalteu) - Fisher
sodium carbonate - Canlab (Baker)
sodium tartrate - Fisher

B. Diluent Preparations

Bi. Insulin diluent composition

sodium acetate - Fisher
sodium chloride - Fisher
zinc chloride - Fisher

Bii. Phenobarbital diluent composition

ethanol - Commercial Alcohols
propylene glycol - Fisher

C. Drug suppliers

atropine - Sigma
2-deoxyglucose - Sigma
dieldrin (99% pure) - Chem Service
glucose - Fisher
insulin (semi-lente, beef and pork) - Connaught
L-a-methyldopa - Sigma
d,l-propranolol dihydrochloride - Sigma
sodium phenobarbital (May & Baker #R-5) -
Health Protection Branch

D. Supplier Addresses

Aldrich - Aldrich Chemical Co., Milwaukee, Wis.
Canlab - Canadian Laboratory Supplies., Toronto, Ont.
Connaught - Connaught Laboratories Ltd., Willowdale,
Ont.
Chem Service - Chem Service, West Chester, Pa.
Commercial Alcohols - Commercial Alcohols Ltd.,
Gatineau, P.Q.
Eastman Kodak - Eastman Kodak Co., Rochester, N.Y.
Fisher - Fisher Scientific Co., Montreal, P.Q.
Gas Dynamics - Division of Liquid Carbide Canada.,
Toronto, Ont.
Health Protection Branch - Health and Welfare Canada.,
Ottawa, Ont.
Sigma - Sigma Chemical Co., St. Louis, Miss.

Appendix 2. Method of preparation of diluent for semi-lente insulin.

According to the U.S. pharmacopeia (1975), semi-lente insulin consists of beef and pork insulin (100 U/ml) suspended in a diluent of:

- 0.15 to 0.17 % (w/v) sodium acetate
- 0.65 to 0.75 % (w/v) sodium chloride
- 0.09 to 0.11 % (w/v) methyl paraben

(preservative)

and sufficient zinc (as zinc chloride) to render the solid phase of the suspension amorphous. Furthermore, according to the Compendium of Pharmaceuticals and Specialties (1981), semi-lente insulin as prepared by Connaught Laboratories Ltd. (Willowdale, Ont.) contains 2.0 to 2.5 mg of zinc per 1000 Units of insulin, and the pH of this preparation lies between 7.1 and 7.5 pH units.

Consequently, the insulin diluent as prepared for use in this study consisted of;

- 0.150 % (w/v) sodium acetate
- 0.700 % (w/v) sodium chloride
- and 0.023 % (w/v) zinc chloride

The diluent was adjusted to a pH of 7.3 with 0.1 N hydrochloric acid. The methyl paraben was omitted as the diluent was prepared fresh daily.

VITA AUCTORIS

Glyn Robert Fox

Born: June 26, 1952
Ottawa, Ontario

Education:

- 1978-83: University of Windsor
Windsor, Ont.
Ph.D.
- 1976-78: University of Victoria
Victoria, B.C.
M.Sc.
- 1970-74: University of Victoria
Victoria, B.C.
B.Sc. (Honours)

Teaching Experience:

- 1978-83: University of Windsor
Human Physiology (1980-83)
Invertebrate Zoology (1979-80)
Introductory Biology (1978-79)
- 1977-78: University of Victoria
Comparative Vertebrate and Invertebrate Physiology

Research Experience:

- 1978-83: Ph.D. dissertation: The Toxicological Significance and Etiology of the Dieldrin (HEOD)-induced Hyperglycemia Response in the Immature and Adult Rat.
- 1976-78: M.Sc. thesis: The Effect of Ethanol with and without Exercise on the Thermal Balance of Man in Cold Water.
- 1970-74: B.Sc. (Honours) thesis: The Postulated Role of Brown Adipose Tissue in the Production of Immunological Incompetence in the Neonatal Rat (Rattus rattus).

Scholarships:

a) Postgraduate Awards

- 1982-83: Ontario Government Graduate Scholarship
- 1982-83: University of Windsor Postgraduate Scholarship
- 1981-82: Ontario Government Graduate Scholarship
- 1981-82: University of Windsor Postgraduate Scholarship
- 1980-81: University of Windsor Postgraduate Scholarship

b) Undergraduate Awards

- 1973-74: British Columbia Government Scholastic Award
- 1970-71: British Columbia Government Scholastic Award

Publications:

a) Abstracts presented to Learned Societies

- Fox, G.R. and B.B. Virgo. 1983. "Neural Control of Dieldrin Induced Hyperglycemia". Proceed. Can. Fed. Biol. Soc. 26: 98.
- Fox, G.R. and B.B. Virgo. 1982. "Dieldrin (HEOD) Toxicity; Significance of Carbohydrate Response". Proceed. Can. Fed. Biol. Soc. 25: 47.
- Fox, G.R. and B.B. Virgo. 1981. "Metabolic Responses to Dieldrin (HEOD) in the Developing Rat". Symposium. Proceed. - Toxic Substances in the Environment. Queens University. Kingston, Ontario. Dec. 12-13th. page 1066.

b) Papers

- Cupples, W.A., G.R. Fox and J.S. Hayward. 1980. "Effect of Cold Water Immersion and Its Combination with Alcohol Intoxication on Urine Flow Rate of Man". Can. J. Physiol. & Pharmacol. 58: 319-322.
- Fox, G.R., J.S. Hayward and G.N. Hobson. 1979. "Effect of Alcohol on Thermal Balance of Man in Cold Water". Can. J. Physiol. & Pharmacol. 57: 860-865.